FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2013/2014

Andreia Gomes Ferreira de Carvalho

Liver Transplantation in Transthyretin Amyloidosis: issues and challenges

março, 2014

FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Andreia Gomes Ferreira de Carvalho

Liver Transplantation in Transthyretin Amyloidosis: issues and challenges

Mestrado Integrado em Medicina

Área: Medicina

Trabalho efetuado sob a Orientação de:

Prof.ª Doutora Luísa Maria Correia Lopes Lobato

Trabalho organizado de acordo com as normas da revista:

Hepato-Gastroenterology

março, 2014

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, Andreia Gomes Ferreira de Carvalho, abaixo assinado, nº mecanográfico 080801039, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 20/03/2014

Assinatura conforme cartão de identificação:

Andreia Gomes Ferreira de Carvalho

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO
UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

Andreia Gomes Ferreira de Carvalho

CARTÃO DE CIDADÃO OU PASSAPORTE (se estrangeiro)	E-MAIL	TELEFONE OU TELEMÓVEL
13752398	mimed08039@med.up.pt	916519700

NÚMERO DE ESTUDANTE	DATA DE CONCLUSÃO
080801039	2014

DESIGNAÇÃO DA ÁREA DO PROJECTO

Medicina

TÍTULO MONOGRAFIA

Liver Transplantation in Transthyretin Amyloidosis: issues and challenges

ORIENTADOR

Prof.ª Doutora Luísa Maria Correia Lopes Lobato

COORIENTADOR (se aplicável)

É autorizada a reprodução integral desta Monografia para efeitos de investigação e de divulgação pedagógica, em programas e projectos coordenados pela FMUP.

Faculdade de Medicina da Universidade do Porto, 20/03/2014

Assinatura conforme cartão de identificação: Andreia Gomes Ferreira de Carvalho

Liver Transplantation in Transthyretin Amyloidosis: issues and challenges

Authors

Andreia Carvalho[1], Ana Rocha[2,3], Luísa Lobato[2,3,4]

Institutional affiliations

[1]Faculty of Medicine, University of Porto, Alameda Prof. Hernâni Monteiro
4200-319, Porto, Portugal

[2]Department of Nephrology, Hospital de Santo António, Centro Hospitalar do Porto
Largo Prof. Abel Salazar, 4099-001, Porto, Portugal

[3]Unidade Corino de Andrade, Hospital de Santo António, Centro Hospitalar do Porto
Rua D. Manuel II, CICAP, 4050-345, Porto, Portugal

[4]Multidisciplinary Unit for Biomedical Research UMIB, Instituto de Ciências Biomédicas
Abel Salazar, University of Porto, Rua Jorge Viterbo Ferreira, 228, 4050-313, Porto, Portugal

Correspondence Luísa Lobato
Department of Nephrology, Hospital de Santo António, Centro Hospitalar do Porto
Largo Prof. Abel Salazar, 4099-001, Porto, Portugal
Phone: + 351 222 074 684; Fax: + 351 222 059 125
lmlobato@icbas.up.pt

E-mail addresses

Andreia Carvalho andreia.gfcarvalho@gmail.com
Ana Rocha acrisbraga@gmail.com
Luísa Lobato lmlobato@icbas.up.pt

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

SUMMARY

Hereditary transthyretin amyloidosis (ATTR) is a rare worldwide autosomal dominant disease caused by a single amino acid substitution in the transthyretin (TTR) gene. The most common mutation is V30M, typical of the Portuguese patients. Each variant has a different involvement, although peripheral neuropathy and cardiomyopathy are the most common. Liver transplantation was implemented as the inaugural disease-modifying therapy since the liver produces the circulating unstable TTR.

In this review, we focus the results and long-term outcomes of liver transplantation in ATTR after 2063 procedures and 23 years of experience. Nerve and organ impairment did not usually reverse after successful transplantation and, in some cases, the disease progressed. Mortality and morbidity are related with later-onset particularly in males, malnutrition and cardiomyopathy. The mutation type, non-TTR V30M, and deposits composed by a mixture of truncated and full-length TTR are associated with lower survival. A higher incidence of early hepatic artery thrombosis of the graft was also documented. The sequential procedure, ATTR livers reused in patients with liver disease, demonstrated that de novo ATTR amyloidosis may appear earlier than expected.

Long-term results of trials with amyloid protein stabilizers or disrupters, silencing-RNA and antisense oligonucleotides will highlight the value and limitations of liver transplantation.

Keywords: transthyretin, amyloid, liver transplant, neuropathy, cardiomyopathy.

INTRODUCTION

Transthyretin (TTR) is a soluble plasmatic transporter of thyroxine and retinol-binding protein. It presents a native tetrameric structure that, in the presence of an amyloidogenic mutation, is more unstable and prone to dissociation into monomers. These are susceptible to misfolding and self-aggregation in insoluble amyloid fibrils capable of systemic extracellular deposition [1,2]. Usually amyloidosis is not evident until adulthood, probably reflecting the influence of non-genetic factors, such as those associated with the ageing process [3]. There is a greater susceptibility to fibrinogen glycation, impairing its chaperone activity and therefore the TTR tetramer stability [4]. To date, more than 120 mutations of the single gene encoding TTR are described in the literature [2,3] and approximately 13 are non-amyloidogenic [5,6].

The disease phenotype is variable according to the genotype, although a clear genotypic/phenotypic relationship is absent [1,6]. The worldwide most common mutation, TTR V30M - substitution of methionine for valine at position 30, has main foci in Portugal, Sweden and Japan [1,7]. It predominantly gives rise to an autosomal-dominant neuropathy first described by Corino de Andrade as Portuguese type Familial Amyloidotic Polyneuropathy (TTR-FAP) [6,8]. This sensorimotor polyneuropathy usually begins with sensitive disorders in the lower extremities, like dissociated anesthesia and paresthesia, which show an upward progression. The autonomic involvement also manifests early, by gastrointestinal disturbances, orthostatic hypotension, neurogenic bladder or erectile dysfunction. Motor dysfunction commonly occurs within a few years [1,8]. Although, other organs such as heart, gastrointestinal tract and kidneys can be affected, demonstrating the disease systemic nature [1,6]. TTR-FAP relentlessly progresses and becomes extremely disabling, with patients losing their walking capacity, experiencing malnutrition, life-threatening autonomic dysfunction and finally death [6]. Familial Amyloidotic Cardiomyopathy is another relatively common phenotype of hereditary TTR amyloidosis (ATTR), characterized by an infiltrative cardiomyopathy with involvement of the conduction system, and usually associates with some non-TTR V30M mutations [1,3,7].

The diagnosis is based on identification of TTR mutation, suggestive clinical picture and histological evidence of TTR amyloid deposits, since ATTR has incomplete penetrance. The definitive diagnosis is established by tissue biopsy, however this has a significant risk of false-negative outcomes owing to the patchy pattern of amyloid deposition [1,6]. In non-endemic areas the diagnosis can be even more difficult once the ATTR generally manifests without familial antecedents [1,6,7] and possibly by atypical features like ataxia or upper limb onset neuropathy [9]. Distinct geographic groups vary in penetrance, age at onset, symptoms and survival, even for the same TTR mutation [1,2,6]. Early-onset ATTR V30M usually occurs in

Portuguese and Japanese patients around the third decade of life; onset in the fifth decade is typical in Swedish cases. The bimodal age at onset may be correlated with the composition of TTR fibrils [2,6,10] and the presence of anti-TTR V30M antibodies [9].

Liver transplantation suppresses the production of circulating mutant TTR and theoretically stops the amyloid formation and disease progression, since most of TTR has hepatic origin [2,6]. Epithelial cells of the choroid plexus and the retinal pigment epithelium also express TTR. Therefore, several genotypes may have leptomeningeal and ocular involvement like vitreous opacities, probably due to locally produced TTR [1,3].

In this review, we focus the current knowledge about orthotopic liver transplantation (OLT). It also addresses combined liver-heart/kidney transplantation, the controversies around sequential liver transplantation and novel therapeutic approaches for ATTR.

ORTHOTOPIC LIVER TRANSPLANTATION FOR HEREDITARY TRANSTHYRETIN AMYLOIDOSIS

Historical Perspective

The inaugural OLT for ATTR was performed in 1990 [11]. Previously the disease management was based only in symptomatic treatment without any improvement in overall survival. Thus, OLT was expected to be a disease-modifying and curative procedure [1,12].

By the time of the first Workshop in Liver Transplantation for FAP (1993), OLT became an accepted treatment for TTR-FAP, since the outcomes were encouraging [11,13]. According to FAP World Transplant Registry (FAPWTR, http://www.fapwtr.org), until 31 December 2012, a total of 2063 patients with ATTR underwent OLT, performed in 77 centers from 19 countries.

Prognostic predictors after orthotopic liver transplantation

After 23 years of cumulative experience is consensual that the major prognostic factors are:

Age at liver transplantation - advanced age is an independent risk factor for late mortality [14-17]. Late-onset disease tends to be associated with lower post-transplant survival [18] and with progressive cardiomyopathy, especially in males with TTR V30M [19]. This seems to be related with age-dependent modifications in extracellular matrix components and deposition of a different type of amyloid fibrils [20].

Duration of disease - OLT performed in long-standing disease (≥7 years) is associated with inferior survival and quality of life (QOL) [6,17,21-25].

Pre-transplant clinical status - peripheral neuropathy does not seem to be correlated with mortality [14,26,27]. It has main impact in QOL since the pre-transplant symptoms usually do not regress significantly [5,6,28]. Autonomic dysfunction however increases the surgical risk

due to intraoperative circulatory instability and may be associated with late sudden death, probably potentiated by life-threatening arrhythmias [6,14,29,30]. In Portuguese type patients, myocardial sympathetic denervation assessed by 123-iodine metaiodobenzylguanidine (^{123}I-MIBG) imaging is an independent prognostic predictor. Late heart-to-mediastinum ^{123}I-MIBG uptake ratio <1.60 is associated with higher neurological disability and mortality, although they apparently still benefit from OLT [31]. Okamoto et al. [32] propose a possible correlation between pre-transplant cardiomyopathy and post-transplant survival, with 10-years survival rates of 92% and 64%, respectively for patients without and with cardiomyopathy.

Preoperative nutritional status - severe malnutrition is associated with higher mortality and OLT does not improve the prognosis [22]. Modified body mass index (mBMI) is remarkable in predicting post-transplant morbidity and survival that is better in patients with mBMI >600, compared to patients not undergoing OLT [18]. mBMI shows a strong correlation with duration of disease and, as an objective measure, allows more accurately survival comparisons [14].

Type of transthyretin mutation - OLT performed for/in non-TTR V30M cases is associated with lower 5-year survival rate [33]. There are also several records of amyloidosis progression in these patients [19,30,34,35].

Composition of transthyretin fibrils - patients with a mixture of truncated and full-length TTR (type A) appear to be more prone to post-transplant development or progression of cardiomyopathy and heart failure, compared to the ones with type B fibrils (only full-length TTR). Hereafter, this histopathological criterion can assume a relevant role in patient selection [36].

Despite all of these predictive factors for OLT outcomes and their importance in optimizing patient selection, establish the prognosis for a specific patient is still a challenge.

Indications and timing of referral for orthotopic liver transplantation

Patients with symptomatic TTR-FAP are the most common candidates for OLT. Asymptomatic carriers have no indication to OLT, since they may never develop the disease [1,6,29]. Mildly symptomatic patients may be deprived of living a few years with good QOL when submitted to OLT since it implies specific cares, like immunosuppression [18].

After clinical stabilization with symptomatic treatment, a selection of transplant candidates should be done according to the prognostic factors mentioned [1]. Suhr et al. [29] defined some unsuitable candidates for OLT - patients with severe neuropathy, particularly if they have hand dysfunction or pronounced risk for circulatory instability; cases of cardiomyopathy with late-onset or in elderly, especially if male, or in patients with type A

fibrils; patients with long-standing disease and mBMI <600. Moreover, OLT seems to be an inadequate treatment for patients with an oculoleptomeningeal phenotype [37-39].

To optimize post-transplant survival and QOL, the referral for OLT should be done immediately after the diagnosis establishment and ideally in initial phases of the disease, particularly stage I (walking unaided) and sometimes early stage II disease (walking with assistance) [1,6,21-24,40]. However, the challenge of early diagnosis and the prolonged waiting time on the transplant list, can be an obstacle to this goal. Besides that, the rate of symptomatic progression is unpredictable due to high inter-individual variability [1,6,14].

Outcomes of orthotopic liver transplantation

Overall patient survival rate: Natural average life expectancy is 9-13 years after the symptomatic presentation and death usually is secondary to cardiac involvement or cachexia [6,10]. The value of OLT in prolonging survival beyond that expected from the natural course of ATTR was demonstrated [16,41]. Five-year survival figures evolved from 78% in 2001 [42] to more than 90% [29] or even 100% [7] at experienced centers. Additionally there are more reports of excellent survival with a 10-year survival estimated probability of 100% compared to 56,1% in non-transplanted patients [41]. These results are probably the reflection of accumulated experience, better patient selection, earlier referral to OLT, recognition of the peculiarity of these patients, prevention and management of intraoperative and posttransplant specific complications [14,17]. However, these statistics are mostly related to patients with ATTR V30M. According to FAPWTR [33], the 5-year survival rate was 59% for non-ATTR V30M patients against 82% for V30M.

Clinicopathological outcomes: Early experience suggested symptomatic improvement as the rule [13,21,43,44], but nowadays it is consensual that the natural steadily clinical deterioration is halted in most of transplanted patients. Therefore, main pre-transplant symptoms will still be present after transplantation [14,17,24,35,40,45,46]. Although some patients may manifest symptomatic improvement or deterioration [12,17,29,47]. The latter is most likely in patients presenting poor prognostic factors [12]. Results vary in accordance with the specific symptoms evaluated (table 1), like cardiovascular manifestations that tend to progress [30]. It has been hypothesized that there is a point of progression of the disease from which there are no regression, possibly because of irreversible organ injury, reinforcing the need for early transplantation [1,35,48].

After OLT, mutant TTR is rapidly cleared from circulation [11], since it has a brief plasma half-life (2.1 days) [44] and there is no consistent evidence that extra-hepatic sources of TTR contribute to its plasmatic pool [2]. Regression of visceral amyloid deposits, evidenced

by serum amyloid P component scintigraphy and acompained by symptomatic improvement, was seen in 2 of the first transplanted patients [13], but without histological evidence of it. In a series of 6 transplanted patients with ATTR V30M, all showed reduction or disappearance of amyloid deposits in sequential abdominal fat aspirates more than 10 years after OLT, even in a patient with disease progression [47]. Remains the question regarding which is the amyloid status of the main target organs. Currently it is believed that the most probable post-transplant outcome is no significant reduction of amyloid deposits [1,45,49], with some reports of ongoing amyloid deposition, essentially at cardiac tissue (Figure 1) and in cases of non-ATTR V30M [12,19,24,30,34,50-52]. Although less significant, there may be amyloidosis progression in other organs [52] (Table 1).

As evidenced by senile systemic amyloidosis, an age-related TTR amyloidosis predominantly manifested by cardiomyopathy, wild-type TTR is capable of amyloid deposition, demonstrating an inherent amyloidogenic capacity [2,6,10,35]. Thus, amyloidogenesis can persist even in the absence of mutant TTR, apparently by deposition of wild-type TTR on a substrate of pre-existing amyloid fibrils [2,6,47,53], especially with type A fibrils [36].

Opposite to end-stage liver disease, the post-transplant improvement is less recognizable in patients with ATTR since they usually have fewer pre-transplant symptoms and this major surgery does not guarantee their regression. The patient must be advised that the aim of OLT is prevent additional deterioration and new complications [6,17,48].

Quality of life: As this is a progressively disabling disease, the goal of OLT goes beyond the mortality reduction. However, this has fallen short of expectations when comparing to liver transplanted patients for other causes, probably due to post-transplant persistence of symptoms [54,55].

Cause of death: In the OLT era, death from cardiac disease acquired greater representation comparatively to historical causes of death like cachexia, infections or cardiovascular collapse [8]. Recent data from FAPWTR points out for 45% of post-transplant deaths from cardiac causes or septicemia. To prevent this outcome, post-transplant follow-up of all ATTR cases, especially in aging patients due to their greater risk for cardiomyopathy progression, must include a periodic echocardiogram and Holter ECG [17,35,38,51]. Okamoto et al. [56] also proposed serial evaluations of serum BNP. ATTR patients are more susceptible to early post-transplant thrombotic complications, in particular to hepatic artery thrombosis that correlates with high morbidity [57].

Sequential Liver Transplantation

Also known as domino liver transplantation (DLT), it consists in transplanting the explanted ATTR liver into a selected non-ATTR patient. This is feasible since ATTR gathers the essential conditions: the liver is anatomically and functionally normal, despite being the major source of mutant TTR, with scarce amyloid deposits and circumscribed to portal vessels and hilar nerves (Figure 2) [58]; has a long latent time before the clinical onset, at least 20 years [59]. Furthermore, as a living graft, the ischemic time is very short and thus the likelihood of graft dysfunction decreases [60].

It was first performed in 1995 in Portugal, by Furtado et al. [59], in order to face the disproportionate supply of deceased organ donors and the rising waiting list for OLT. The initial DLT recipients were patients with unresectable primary or metastatic liver cancers, with no extrahepatic spread and short life expectancy. The safety and successful short-term results for both DLT donors and recipients allowed a worldwide acceptance [59-61]. The last update of Domino Liver Transplant Registry (DLTR) displays a total of 1085 DLT performed, with ATTR patients as the main donors.

However, the main pitfall of DLT is the risk of the recipient develops de novo ATTR, since circulating mutant TTR is soon after detected. Thus the need for a close follow-up with monitorization of clinical signs was early recognized [58,59,61].

Despite the hope that the disease would take at least 30 years to develop, based on the natural history of ATTR [59], the first report of symptomatic systemic TTR amyloidosis in a DLT recipient occurred after 8 years of follow-up [62]. However, subclinical skin and nerve amyloid deposits were found 3 and 6 years after DLT, respectively [63]. Electrophysiological signs of progressive peripheral polyneuropathy without clinical symptoms were also detected 2-5 years after DLT, however the definitive diagnosis by nerve biopsies couldn't be established [64].

Recently, cases of sensory neuropathy of the lower limbs without autonomic involvement, occurring as early as 6-7 years after transplant, have been published [65-70]. Possible explanations for this accelerated amyloidogenesis are related to age factors since the clinical onset in the cases mentioned varied between 55 and 75 years. However, Obayashi et al. [70] described a case of TTR amyloidosis in a 45-year-old man with primary sclerosing cholangitis that underwent DLT 10 years before. Other potential justifications are surgical trauma, immunosuppression and inflammatory reactions related to graft rejection or reactivation of latent viruses [71]. The clinical onset of TTR amyloidosis in DLT recipients has generally been considered an indication for retransplantation with a non-ATTR liver [61,62,64].

Its efficacy in halting the disease progression is now starting to be reported, with one case of partial symptomatic recovery [72]. Futurely, pharmacologic treatment may also have a role.

Ericzon et al. [71] advocates that DLT should continue since the ATTR liver can still be an excellent graft when a case by case risk-benefit assessment is done. Other experts [66-68,70] reinforce this idea and the need for the future DLT recipient to give a truly informed consent and to be regularly monitored for de novo TTR amyloidosis. Recently, Bolte et al. [69] proposed a scheme for neurologic follow-up of these patients based on periodic assessment of specific scores of peripheral neuropathy, quantitative sensory testing, nerve conduction studies and sural nerve biopsy performed only in patients with ≥2 alterations in the previous tests. According to these findings and the possibility of neuropathy from other etiologies, an estimate of the relative probability of de novo TTR amyloidosis is made.

Multiple Organ Transplantation

According to the FAPWTR, among 2063 reported transplants, there were 46 simultaneous heart-liver transplants and 47 simultaneous liver-kidney transplants. Only 4 were combined heart-liver-kidney transplants.

The first worldwide Combined Heart-Liver Transplantation (CHLT) for ATTR dates back beyond 1993 [73] and the following reports show that all of them were performed in non-TTR V30M cases [74-76]. This can be explained by their higher and earlier predisposition to cardiomyopathy, that can progress even after successful OLT [30]. Thus they represent the main candidates for this procedure [12,74,75]. Outcomes of CHLT have been adversely influenciated by the preoperative clinical status. However, they seem to be encouraging since histological and imagiological evidences of amyloid deposits have not been found yet [74,75]. Nevertheless, the hypothesis of progressive neurological disability after CHLT remains [74].

Combined liver-kidney transplantation (CLKT) is indicated in patients with end-stage renal disease, since renal replacement therapy correlates with poor survival. Moreover, isolated OLT in patients with a preoperative glomerular filtration rate <30mL/min is associated with a significant postoperative deterioration of kidney function [77,78]. A series of 13 patients with different indications for CLKT, including 3 patients with FAP, reinforced the idea that the liver graft may have a protective immunologic effect on the kidney graft [79]. Lobato et al. [77] reported the absence of proteinuria and a mean serum creatinine in the superior limit of the reference interval after 84 months of follow-up of 6 patients with ATTR V30M submitted to CLKT. These results suggest that CLKT prevents the recurrence of nephropathy, although without histologic evidence of it, but not the progression of polyneuropathy, as expected.

NOVEL THERAPEUTIC APPROACHES

The persistent searching for an ideal therapy to ATTR began too many years ago and it still continues today with a preference towards less invasive options, since the current ones have imperfections or need more time of experience to evaluate the long-term outcomes.

The partial understanding of TTR amyloidogenesis has allowed the development of several potencial new treatments acting in different phases of the process (Table 2). Further progresses in that area are expected due to recent success in generation of ATTR-specific induced pluripotent stem cells [80]. Nowadays the most promising drugs seem to be the TTR stabilizing agents. They prevent amyloidogenesis by inhibiting the TTR tetramer dissociation and include the first medical treatment specifically approved to TTR-FAP in Europe in 2011 - tafamidis meglumine. This drug has shown capacity to slow down the progression of early stages of peripheral neuropathy and therefore is formally indicated for patients with the neuropathic form of ATTR in stage I, regardless of the type of mutation [1,10]. Nevertheless there is evidence that suggests less efficacy in halting neurological dysfunction in non-TTR V30M mutations. Patients with late-onset ATTR V30M involved in a non-randomized controlled trial showed disability progression in 55% of cases and increased Neuropathy Impairment Scores in most of them [9]. Nonsteroidal anti-inflammatory drugs (NSAIDs) are capable of stabilize the TTR tetramer since their structure resembles the one of thyroxine, that is the main responsible for tetramer stabilization. Diflunisal is already in clinical trials with the actual concerns being the therapeutic serum concentrations, the adverse effects profile and its efficacy in other phenotypes of ATTR [1,10]. An open-label uncontrolled trial was conducted with 13 patients presenting symptomatic and biopsy-proven ATTR cardiomyopathy. They were medicated with diflunisal (250mg bid) for a mean of 10.8 months, during which no significant changes in cardiac structure function and biomarkers were observed. Diflunisal seems to have potential to slow the disease progression, although needs careful renal and hematologic monitoring. These results require confirmation in a randomized placebo-controlled trial [81].

Several agents are presently under investigation, with some yet restricted to preclinical studies. A recent article about clinical management of ATTR patients expresses the idea that as new effective drugs become available they should be offered to patients meeting their clinical indications, irrespective of liver transplant plans [1].

DISCUSSION

More than half century after the first description of this progressively incapacitating disease there are still some challenges and unresolved issues, particularly at therapeutic level.

After 23 years of accumulated experience it is well-known that OLT, the first disease-modifying therapy, allowed a revolution in ATTR treatment, though it has some limitations. In the majority of patients the preexisting symptoms will remain unchanged after OLT and besides, it is incapable of alter the natural course of oculoleptomeningeal amyloidosis. Not all patients with ATTR are suitable candidates for OLT, therefore the outcomes of this procedure have been optimized through a careful patient selection. However is still something missing since there are some reports of post-transplant disease progression. Amyloid fibril composition may add some insight in outcome prediction. The shortage of liver donors is another important restriction, partially relieved by DLT that recently has shown some unexpected unfavorable long-term outcomes. There is a need for better understanding of possible predisposing factors to systemic TTR amyloidosis in DLT recipients, like early-onset disease or non-TTR V30M mutations in DLT donors. Furthermore, OLT is an invasive therapy with significant surgical risk and requires long-standing immunosuppression. Consequently, OLT is not approved for asymptomatic patients with TTR amyloid deposits (stage 0 of disease).

Current therapeutic options for ATTR are still limited, but recently expanded with another disease-modifying therapy - tafamidis. The approval of this drug in Europe has raised several unanswered questions such as its efficacy in the treatment of amyloid cardiomyopathy and for patients with stage 0 or advanced stages of disease, for which there is still a gap in therapeutic options. The future may validate a combined approach to ATTR, OLT plus drug treatment, but for now is still controversial if patients showing improvement under tafamidis should remain on the transplant list. Moreover, the hope also rests on the approval of new drugs, like the ones capable of amyloid deposits dissolution.

Is liver transplantation really a life-saving treatment for ATTR? The truth is that OLT, besides all the controversies and limitations, has allowed a revolutionary change in survival of most patients, but the future about curative treatments is still unwritten.

ACKNOWLEDGMENTS

The authors thank to clinicians from Unidade Corino de Andrade of Hospital de Santo António, Centro Hospitalar do Porto, Portugal. Multidisciplinary Unit for Biomedical Research is funded by grants from Foundation for Science and Technology (Fcomp-01-0124-FEDER-015893).

REFERENCES

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

2) **Benson MD:** Liver transplantation and transthyretin amyloidosis. Muscle Nerve 2013; 2:157-162.

3) **Benson MD, Kincaid JC:** The molecular biology and clinical features of amyloid neuropathy. Muscle Nerve 2007; 4:411-423.

4) **da Costa G, Gomes RA, Guerreiro A, et al:** Beyond genetic factors in familial amyloidotic polyneuropathy: protein glycation and the loss of fibrinogen's chaperone activity. PLoS One 2011; 10:e24850.

5) **Ando Y, Nakamura M, Araki S:** Transthyretin-related familial amyloidotic polyneuropathy. Arch Neurol 2005; 7:1057-1062.

6) **Suhr OB, Herlenius G, Friman S, et al:** Liver transplantation for hereditary transthyretin amyloidosis. Liver Transpl 2000; 3:263-276.

7) **Araki S, Ando Y:** Transthyretin-related familial amyloidotic polyneuropathy-Progress in Kumamoto, Japan (1967-2010). Proc Jpn Acad Ser B Phys Biol Sci 2010; 7:694-706.

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

9) **Adams D, Theaudin M, Cauquil C, et al:** FAP neuropathy and emerging treatments. Curr Neurol Neurosci Rep 2014; 3:435.

10) **Hund E:** Familial amyloidotic polyneuropathy: current and emerging treatment options for transthyretin-mediated amyloidosis. Appl Clin Genet 2012; 37-41.

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

12) **Sharma P, Perri RE, Sirven JE, et al:** Outcome of liver transplantation for familial amyloidotic polyneuropathy. Liver Transpl 2003; 12:1273-1280.

13) **Holmgren G, Ericzon BG, Groth CG, et al:** Clinical improvement and amyloid regression after liver transplantation in hereditary transthyretin amyloidosis. Lancet 1993; 8853:1113-1116.

14) **Suhr OB, Ericzon BG, Friman S:** Long-term follow-up of survival of liver transplant recipients with familial amyloid polyneuropathy (Portuguese type). Liver Transpl 2002; 9:787-794.

15) **Juneblad K, Naslund A, Olofsson BO, et al:** Outcome of exercise electrocardiography in familial amyloidotic polyneuropathy patients, Portuguese type, under evaluation for liver transplantation. Amyloid 2004; 3:208-213.

16) **Okamoto S, Wixner J, Obayashi K, et al:** Liver transplantation for familial amyloidotic polyneuropathy: impact on Swedish patients' survival. Liver Transpl 2009; 10:1229-1235.

17) **Yamamoto S, Wilczek HE, Nowak G, et al:** Liver transplantation for familial amyloidotic polyneuropathy (FAP): a single-center experience over 16 years. Am J Transplant 2007; 11:2597-2604.

18) **Suhr OB, Friman S, Ericzon BG:** Early liver transplantation improves familial amyloidotic polyneuropathy patients' survival. Amyloid 2005; 4:233-238.

19) **Okamoto S, Zhao Y, Lindqvist P, et al:** Development of cardiomyopathy after liver transplantation in Swedish hereditary transthyretin amyloidosis (ATTR) patients. Amyloid 2011; 4:200-205.
20) **Koike H, Hashimoto R, Tomita M, et al:** Impact of aging on the progression of neuropathy after liver transplantation in transthyretin Val30Met amyloidosis. Muscle Nerve 2012; 6:964-970.
21) **Suhr OB, Holmgren G, Steen L, et al:** Liver transplantation in familial amyloidotic polyneuropathy. Follow-up of the first 20 Swedish patients. Transplantation 1995; 9:933-938.
22) **Suhr OB, Ando Y, Holmgren G, et al:** Liver transplantation in familial amyloidotic polyneuropathy (FAP). A comparative study of transplanted and non-transplanted patient's survival. Transpl Int 1998; S160-163.
23) **Jonsen E, Suhr OB, Tashima K, et al:** Early liver transplantation is essential for familial amyloidotic polyneuropathy patients' quality of life. Amyloid 2001; 1:52-57.
24) **Bittencourt PL, Couto CA, Farias AQ, et al:** Results of liver transplantation for familial amyloid polyneuropathy type I in Brazil. Liver Transpl 2002; 1:34-39.
25) **Franz C, Hoffmann K, Hinz U, et al:** Modified body mass index and time interval between diagnosis and operation affect survival after liver transplantation for hereditary amyloidosis: a single-center analysis. Clin Transplant 2013; 40-48.
26) **Suhr O, Danielsson A, Rydh A, et al:** Impact of gastrointestinal dysfunction on survival after liver transplantation for familial amyloidotic polyneuropathy. Dig Dis Sci 1996; 10:1909-1914.
27) **Parrilla P, Ramirez P, Andreu LF, et al:** Long-term results of liver transplantation in familial amyloidotic polyneuropathy type I. Transplantation 1997; 4:646-649.
28) **Ando Y:** Liver transplantation and new therapeutic approaches for familial amyloidotic polyneuropathy (FAP). Med Mol Morphol 2005; 3:142-154.
29) **Suhr OB, Ericzon BG:** Selection of hereditary transthyretin amyloid patients for liver transplantation: the Swedish experience. Amyloid 2012; 78-80.
30) **Pomfret EA, Lewis WD, Jenkins RL, et al:** Effect of orthotopic liver transplantation on the progression of familial amyloidotic polyneuropathy. Transplantation 1998; 7:918-925.
31) **Coutinho MC, Cortez-Dias N, Cantinho G, et al:** Reduced myocardial 123-iodine metaiodobenzylguanidine uptake: a prognostic marker in familial amyloid polyneuropathy. Circ Cardiovasc Imaging 2013; 5:627-636.
32) **Okamoto S, Wixner J, Ericzon BG, et al:** Prognostic value of pre-transplant cardiomyopathy in Swedish liver transplanted patients for familial amyloidotic polyneuropathy. Amyloid 2011; 171-173.
33) **Wilczek HE, Larsson M, Ericzon BG:** Long-term data from the Familial Amyloidotic Polyneuropathy World Transplant Registry (FAPWTR). Amyloid 2011; 193-195.
34) **Garcia-Herola A, Prieto M, Pascual S, et al:** Progression of cardiomyopathy and neuropathy after liver transplantation in a patient with familial amyloidotic polyneuropathy caused by tyrosine-77 transthyretin variant. Liver Transpl Surg 1999; 3:246-248.
35) **Suhr OB:** Impact of liver transplantation on familial amyloidotic polyneuropathy (FAP) patients' symptoms and complications. Amyloid 2003; 77-83.
36) **Gustafsson S, Ihse E, Henein MY, et al:** Amyloid fibril composition as a predictor of development of cardiomyopathy after liver transplantation for hereditary transthyretin amyloidosis. Transplantation 2012; 10:1017-1023.

37) **Munar-Ques M, Salva-Ladaria L, Mulet-Perera P, et al:** Vitreous amyloidosis after liver transplantation in patients with familial amyloid polyneuropathy: ocular synthesis of mutant transthyretin. Amyloid 2000; 4:266-269.
38) **Ohya Y, Okamoto S, Tasaki M, et al:** Manifestations of transthyretin-related familial amyloidotic polyneuropathy: long-term follow-up of Japanese patients after liver transplantation. Surg Today 2011; 9:1211-1218.
39) **Sandgren O, Kjellgren D, Suhr OB:** Ocular manifestations in liver transplant recipients with familial amyloid polyneuropathy. Acta Ophthalmol 2008; 5:520-524.
40) **Tashima K, Ando Y, Terazaki H, et al:** Outcome of liver transplantation for transthyretin amyloidosis: follow-up of Japanese familial amyloidotic polyneuropathy patients. J Neurol Sci 1999; 1:19-23.
41) **Yamashita T, Ando Y, Okamoto S, et al:** Long-term survival after liver transplantation in patients with familial amyloid polyneuropathy. Neurology 2012; 9:637-643.
42) **Herlenius G, Larsson M, Ericzon BG:** Results from the familial amyloidotic polyneuropathy world transplant registry. Transplant Proc 2001; 4:2454.
43) **Bergethon PR, Sabin TD, Lewis D, et al:** Improvement in the polyneuropathy associated with familial amyloid polyneuropathy after liver transplantation. Neurology 1996; 4:944-951.
44) **Ando Y, Tanaka Y, Nakazato M, et al:** Change in variant transthyretin levels in patients with familial amyloidotic polyneuropathy type I following liver transplantation. Biochem Biophys Res Commun 1995; 2:354-358.
45) **Anan I, El-Salhy M, Nyhlin N, et al:** Liver transplantation restores endocrine cells in patients with familial amyloidotic polyneuropathy. Transplantation 2000; 5:794-799.
46) **Shimojima Y, Morita H, Kobayashi S, et al:** Ten-year follow-up of peripheral nerve function in patients with familial amyloid polyneuropathy after liver transplantation. J Neurol 2008; 8:1220-1225.
47) **Tsuchiya A, Yazaki M, Kametani F, et al:** Marked regression of abdominal fat amyloid in patients with familial amyloid polyneuropathy during long-term follow-up after liver transplantation. Liver Transpl 2008; 4:563-570.
48) **Lang K, Wikstrom L, Danielsson A, et al:** Outcome of gastrointestinal complications after liver transplantation for familial amyloidotic polyneuropathy. Scand J Gastroenterol 2000; 9:985-989.
49) **Nyhlin N, Anan I, El SM, et al:** Reduction of free radical activity in amyloid deposits following liver transplantation for familial amyloidotic polyneuropathy. J Intern Med 2002; 2:136-141.
50) **Munitiz V, Ramirez P, Munar M, et al:** Reversibility of the neurologic alterations in familial amyloidotic polyneuropathy type I after liver transplantation (22 cases). Transplant Proc 2002; 1:310-311.
51) **Olofsson BO, Backman C, Karp K, et al:** Progression of cardiomyopathy after liver transplantation in patients with familial amyloidotic polyneuropathy, Portuguese type. Transplantation 2002; 5:745-751.
52) **Yazaki M, Mitsuhashi S, Tokuda T, et al:** Progressive wild-type transthyretin deposition after liver transplantation preferentially occurs onto myocardium in FAP patients. Am J Transplant 2007; 1:235-242.
53) **Liepnieks JJ, Benson MD:** Progression of cardiac amyloid deposition in hereditary transthyretin amyloidosis patients after liver transplantation. Amyloid 2007; 4:277-282.

54) **Drent G, Graveland CW, Hazenberg BP, et al:** Quality of life in patients with familial amyloidotic polyneuropathy long-term after liver transplantation. Amyloid 2009; 3:133-141.
55) **Telles-Correia D, Cortez-Pinto H, Barbosa A, et al:** Quality of life following liver transplantation: a comparative study between Familial Amyloid Neuropathy and liver disease patients. BMC Gastroenterol 2009; 54.
56) **Okamoto S, Yamashita T, Ando Y, et al:** Evaluation of myocardial changes in familial amyloid polyneuropathy after liver transplantation. Intern Med 2008; 24:2133-2137.
57) **Bispo M, Marcelino P, Freire A, et al:** High incidence of thrombotic complications early after liver transplantation for familial amyloidotic polyneuropathy. Transpl Int 2009; 2:165-171.
58) **Shaz BH, Gordon F, Lewis WD, et al:** Orthotopic liver transplantation for familial amyloidotic polyneuropathy: a pathological study. Hum Pathol 2000; 1:40-44.
59) **Furtado A, Tome L, Oliveira FJ, et al:** Sequential liver transplantation. Transplant Proc 1997; 1-2:467-468.
60) **Furtado AJ:** Domino liver transplantation using FAP grafts. HUC experience--hopes and realities. Amyloid 2003; 84-87.
61) **Azoulay D, Samuel D, Castaing D, et al:** Domino liver transplants for metabolic disorders: experience with familial amyloidotic polyneuropathy. J Am Coll Surg 1999; 6:584-593.
62) **Stangou AJ, Heaton ND, Hawkins PN:** Transmission of systemic transthyretin amyloidosis by means of domino liver transplantation. N Engl J Med 2005; 22:2356.
63) **Sousa MM, Ferrao J,Fernandes R, et al:** Deposition and passage of transthyretin through the blood-nerve barrier in recipients of familial amyloid polyneuropathy livers. Lab Invest 2004; 7:865-873.
64) **Yamamoto S, Wilczek HE, Iwata T, et al:** Long-term consequences of domino liver transplantation using familial amyloidotic polyneuropathy grafts. Transpl Int 2007; 11:926-933.
65) **Goto T, Yamashita T, Ueda M, et al:** Iatrogenic amyloid neuropathy in a Japanese patient after sequential liver transplantation. Am J Transplant 2006; 10:2512-2515.
66) **Barreiros AP, Geber C,Birklein F, et al:** Clinical symptomatic de novo systemic transthyretin amyloidosis 9 years after domino liver transplantation. Liver Transpl 2010; 1:109.
67) **Llado L, Baliellas C, Casasnovas C, et al:** Risk of transmission of systemic transthyretin amyloidosis after domino liver transplantation. Liver Transpl 2010; 12:1386-1392.
68) **Samuel D, Adams D:** Domino liver transplantation from familial amyloidotic polyneuropathy donors: how close is the damocles sword to the recipient? Transpl Int 2007; 11:921-923.
69) **Bolte FJ, Schmidt HH, Becker T, et al:** Evaluation of domino liver transplantations in Germany. Transpl Int 2013; 7:715-723.
70) **Obayashi K, Yamashita T, Tasaki M, et al:** Amyloid neuropathy in a younger domino liver transplanted recipient. Muscle Nerve 2011; 3:449-450.
71) **Ericzon BG:** Domino transplantation using livers from patients with familial amyloidotic polyneuropathy: should we halt? Liver Transpl 2007; 2:185-187.
72) **Antonini TM, Lozeron P,Lacroix C, et al:** Reversibility of acquired amyloid polyneuropathy after liver retransplantation. Am J Transplant 2013; 10:2734-2738.
73) **Rela M, Muiesan P, Heaton ND, et al:** Orthotopic liver transplantation for hepatic-based metabolic disorders. Transpl Int 1995; 1:41-44.

74) **Pilato E, Dell'Amore A, Botta L, et al:** Combined heart and liver transplantation for familial amyloidotic neuropathy. Eur J Cardiothorac Surg 2007; 1:180-182.
75) **Barreiros AP, Post F, Hoppe-Lotichius M, et al:** Liver transplantation and combined liver-heart transplantation in patients with familial amyloid polyneuropathy: a single-center experience. Liver Transpl 2010; 3:314-323.
76) **Arpesella G, Chiappini B, Marinelli G, et al:** Combined heart and liver transplantation for familial amyloidotic polyneuropathy. J Thorac Cardiovasc Surg 2003; 5:1165-1166.
77) **Lobato L, Beirao I, Seca R, et al:** Combined liver-kidney transplantation in familial amyloidotic polyneuropathy TTR V30M: nephrological assessment. Amyloid 2011; 190-192.
78) **Lobato L, Rocha A:** Transthyretin amyloidosis and the kidney. Clin J Am Soc Nephrol 2012; 8:1337-1346.
79) **Gonzalez MR, Ramirez P, Cascales P, et al:** Thirteen cases of liver-kidney transplantation. Transplant Proc 2010; 8:3162-3163.
80) **Isono K, Jono H, Ohya Y, et al:** Generation of familial amyloidotic polyneuropathy-specific induced pluripotent stem cells. Stem Cell Res 2014; 2:574-583.
81) **Castano A, Helmke S, Alvarez J, et al:** Diflunisal for ATTR cardiac amyloidosis. Congest Heart Fail 2012; 6:315-319.
82) **Ando Y, Tanaka Y, Ando E, et al:** Effect of liver transplantation on autonomic dysfunction in familial amyloidotic polyneuropathy type I. Lancet 1995; 8943:195-196.
83) **Suhr OB, Wiklund U, Ando Y, et al:** Impact of liver transplantation on autonomic neuropathy in familial amyloidotic polyneuropathy: an evaluation by spectral analysis of heart rate variability. J Intern Med 1997; 3:225-229.
84) **Ohya Y, Isono K, Obayashi K, et al:** Peritransplant gastrointestinal symptoms in familial amyloidotic polyneuropathy. Exp Clin Transplant 2013; 4:327-331.
85) **Rocha A, Lobato L, Silva H, et al:** Characterization of end-stage renal disease after liver transplantation in transthyretin amyloidosis (ATTR V30M). Transplant Proc 2011; 1:189-193.
86) **Sakashita N, Ando Y, Haraoka K, et al:** Severe congestive heart failure with cardiac liver cirrhosis 10 years after orthotopic liver transplantation for familial amyloidotic polyneuropathy. Pathol Int 2006; 7:408-412.
87) **Okamoto S, Hornsten R, Obayashi K, et al:** Continuous development of arrhythmia is observed in Swedish transplant patients with familial amyloidotic polyneuropathy (amyloidogenic transthyretin Val30Met variant). Liver Transpl 2011; 2:122-128.
88) **Snanoudj R, Durrbach A, Gauthier E, et al:** Changes in renal function in patients with familial amyloid polyneuropathy treated with orthotopic liver transplantation. Nephrol Dial Transplant 2004; 7:1779-1785.
89) **Nowak G, Suhr OB, Wikstrom L, et al:** The long-term impact of liver transplantation on kidney function in familial amyloidotic polyneuropathy patients. Transpl Int 2005; 1:111-115.
90) **Hara R, Kawaji T, Ando E, et al:** Impact of liver transplantation on transthyretin-related ocular amyloidosis in Japanese patients. Arch Ophthalmol 2010; 2:206-210.

TABLES

Table 1 - Clinicopathological outcomes		
	Clinical outcomes	**Histopathological outcomes**
Peripheral neuropathy	- Neurologic dysfunction rarely progresses [13,17,27,40,50]. - Sensory disturbances and early stages of disease are more likely to improve [6,17,35]. - Recovery of peripheral nerve function is rare [40,46].	- Regression of amyloid deposits in peripheral nerves is still uncertain [46].
Autonomic neuropathy	- Several reports of autonomic disturbances regression, particularly in ATTR V30M [13,30,40,50,82,83]. - Improvement of orthostatic hypotension [27,43]. - Decrease of gastrointestinal symptoms and improvement in nutritional status [17,21,43,50,84]. - Few reports about bladder and erectile dysfunctions which showed variable outcomes [50,85].	- Contradictory histological evidence, favoring the post-transplant persistence of denervation [40].
Cardiac amyloidosis	- Several reports of cardiomyopathy progression after successful OLT, predominantly but not exclusive for non-ATTR V30M [17,30,34,51,56,86]. - Aging also seems to be a risk factor for progression [38]. - Does not prevent life-threatening arrhythmias, which seem more prevalent in ATTR V30M [38,75,87].	- Autopsy findings showed a predominance of wild-type TTR in post-transplant cardiac amyloid deposits [52,53].
Renal amyloidosis	- Stabilization of kidney function after an initial deterioration - controversial [35,38,88,89]. - Regression, intensification or onset of proteinuria [85]. - Other factors can contribute to renal impairment - nephrotoxicity of immunosuppressants, kidney damage secondary to neurogenic bladder, diabetes mellitus [6,35].	- The rare histopathological reports suggest that OLT can prevent further progression of kidney amyloidosis [38,86,88]. - Biochemical analysis of amyloid kidney deposits supports for *de novo* amyloidogenesis [52].
Ocular amyloidosis	- Progression independently of the type of TTR mutation [37-40,50,90]. - Aging is a major determinant of this course [38].	
Leptomeningeal amyloidosis	- One report of unsuccessful OLT in two siblings with ATTR Leu12Pro manifested by grand mal seizures [75].	

Table 2. Potential therapeutic strategies for ATTR according to the model of TTR amyloidogenesis		
Mechanism	**Potential treatments**	**Considerations**
Substitution of mutant TTR gene for normal TTR gene	**Liver transplantation**[#]	
	Gene therapy (conversion) - Single-stranded oligonucleotides*	Gene therapy to repair the mutant TTR gene has still a long path before it can be applied clinically.
Suppression of TTR mRNA expression	**Injection of a large amount of normal TTR**	Significant (but not enough) reduction in mutant TTR plasmatic levels. Clinical utility precluded by the rapid turnover of TTR.
	Gene therapy (silencing)*: - Small interfering RNA: ALN-TTR01, ALN-TTR02 - Antisense oligonucleotides: ISIS-TTR_{RX}	Synthesis inhibition of mutant and wild-type TTR, both with amyloidogenic capacity. Induces a dose-dependent and more durable response. Phase 3 clinical trials.
Decrease plasmatic levels of mutant TTR	**Plasma exchange**	Ineffective in inducing enough decrease of serum TTR. Infeasible due to short TTR half-life, implying a continuous treatment.
	Affinity column chromatography	
	TTR absorption column chromatography	
Stabilization of the TTR tetramer	**Thyroxine-based therapeutic drugs:** - Tafamidis meglumine (Fx-1006A)[#] - NSAIDs*: diflunisal, diclofenac, flufenamic acid	The most promising agents for halting the relentless progression of ATTR.
	Trivalent chromium (Cr^{3+})*	Can potentiate the effects of NSAIDs due to its non-competitive action.
	TTR T119M monomers*	These non-amyloidogenic monomers are incorporated into tetramers making them less amyloidogenic. *In vitro* studies.
Prevention of amyloid formation	**Free radical scavenger therapy** (300mg N-acetylcysteine, 300mg α-tocopherol and 500mg vitamin C)	Without effect in non-transplanted patients; slight improvement in the nutritional status of transplanted patients.
	Immunization with unstable TTR (TTR Y78F)	Clinical application limited by immunization-related inflammation.
	Cyclodextrin*	Reduces conformational change of TTR. In preclinical studies.
	Carvedilol*	Decreases amyloid deposits due to antioxidant effect. In preclinical studies.
Dissolution of amyloid aggregates	**IDOX** (4'-Iodo-4'-deoxydoxorubicin)	Lack of in vivo studies. Nephrotoxic.
	Doxy-TUDCA (Doxycycline and tauroursodeoxycholic acid)*	Seems to stabilize the disease at least during 1 year. Tolerable adverse effects.
	Anti-SAP (serum amyloid P component) **monoclonal antibodies***	Phase 1 study in patients with AA amyloidosis.
	Epigallocatechin-3-gallate*	In preclinical trials.

[#] Current available therapies for clinical use. * Therapies with promising future outcomes. Based on Ando et al. [1,28], Araki et al. [7], Hund et al. [10], Lobato et al. [78] and Adams et al. [9].

FIGURE LEGENDS

Figure 1 - An example of severe cardiomegaly in a patient with progressive amyloid cardiomyopathy after orthotopic liver transplantation (Luísa Lobato, personal archives).

Figure 2 - Congo red stained material showing amyloid deposits in the arteriole of the liver in a sequential liver receptor from a donor with ATTR V30M-amyloidosis (x400); amyloid spares bile ducts (Luísa Lobato, personal archives).

FIGURE 1

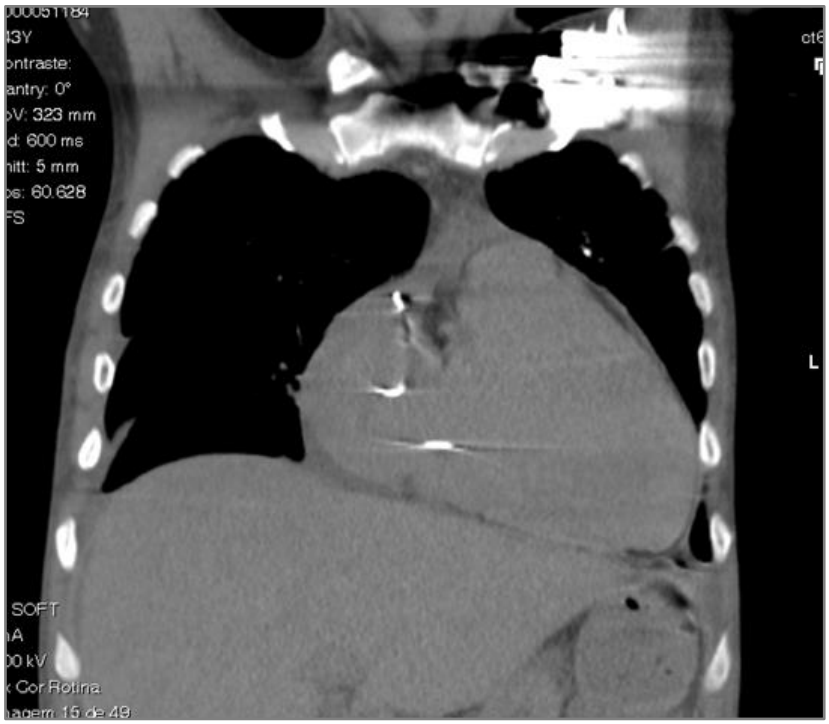
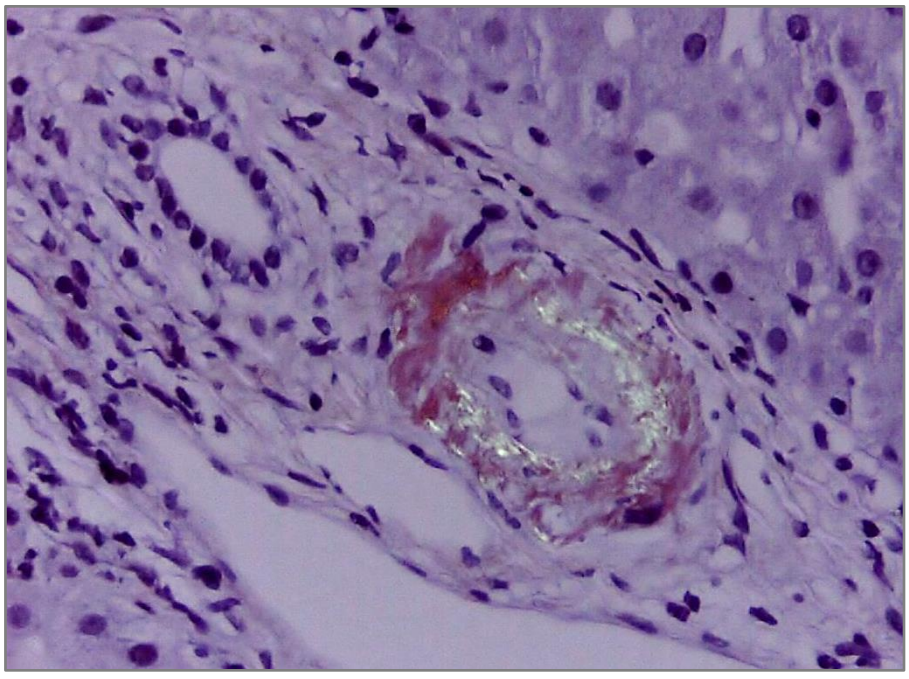


FIGURE 2



Agradecimentos

Aos meus pais e irmãos, o meu eterno alicerce.

À Professora Luísa Lobato, pela orientação, conhecimentos transmitidos, dedicação, disponibilidade e paciência com que sempre me acolheu.

À Drª Ana Rocha, pelas valiosas dicas.

Aos meus amigos, em particular à Olga, que foi incansável e esteve sempre disponível.

- Guidelines for Authors

Guidelines for Authors

General information

Hepato-Gastroenterology publishes work on topics concerning the medical and surgical management of GI diseases, including the exploration of new therapeutic trends. Original, unpublished papers undergo peer review before being accepted for publication. Authors are invited to suggest potential reviewers. However, in all cases, the editorial staff's decision is final.

The journal publishes several types of paper:

• **Original Papers:** presenting the results of full-length studies

Surgical Techniques: outlining new surgical procedures
Literature Reviews
Express Communication
Exceptional quality Case Reports

The Editors also welcome submission of:

• Calendar information regarding GI-related congresses and meetings worldwide.

• Editorial articles.

• News from around the world.

• Feature articles on work/research being carried out in particular geographic locations.

• Feature articles relevant to the field of GI medicine.

Submission process

Manuscripts must be submitted by one of the authors of the manuscript, and should not be submitted by anyone on their behalf. The corresponding author will be contacted with regards to any matters

concerning the submission process and acceptance/rejection of the manuscript.

Hepato-Gastroenterology only accepts manuscripts submitted via this online submission system.

Files can be submitted as a batch, or one by one. The submission process can be interrupted at any time – when users return to the site, they can carry on where they left off.

See below for examples of acceptable word processor and graphics file formats.

During submission you will be asked to provide a cover letter.

Assistance with the process of manuscript preparation and submission is available from our Managing Editor at editor@iasgo.org.

ACCEPTANCE

Acceptance for publication is a two-stage process:

• Initial acceptance is purely on grounds of medical interest and merit.

• Editorial acceptance follows when the paper/article is being prepared for typesetting. Papers must conform absolutely to the regulations below, otherwise they will be returned UNEDITED for revision.

NOTE: To prevent delays in publication, we strongly suggest that papers should be checked carefully for grammatical, lexical and spelling errors **BEFORE** submission, paying particular attention to the formatting style of the journal.

Publication and open access options

Hepato-Gastroenterology is a hybrid open access journal. Authors have the choice of paying an open access fee to have their article published online up to one month following payment, through a provisional PDF (with DOI number) which is accessible to all readers of the **Hepato-Gastroenterology** website. These articles will eventually be printed in hard copy and given an Issue number and page numbers and will be included in PubMed.

Alternatively, authors have the choice of waiting in line to be assigned an Issue number and their manuscript will be published initially online with a provisional PDF (with DOI number) once the Issue number has been assigned, but will be accessible ONLY TO SUBSCRIBERS OF IASGO/ **Hepato-Gastroenterology**. This will eventually be printed in hard copy with Issue number and page numbers and will be included in PubMed.

The Editor-in-Chief has the final right to decide upon rejection or acceptance, to whom any appeals against rejection should be addressed.

Open Access Fees

The authors have the choice to pay the open access fee after acceptance of their article, for immediate publication of their article and free access of this to all readers of **Hepato-Gastroenterology**. In 2011 the

article-processing charge was €1000. This fee is reduced to €700 for members of IASGO for all manuscripts submitted over the duration of their membership.

Ethical policies

Submission of a manuscript to **Hepato-Gastroenterology** implies that all authors have read and agreed to its content, and that any experimental research that is reported in the manuscript has been performed with the approval of an appropriate ethics committee. Research carried out on humans must be in compliance with the Helsinki Declaration, and any experimental research on animals must follow internationally recognized guidelines. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate. Informed consent must also be documented. Manuscripts may be rejected if the editorial office considers that the research has not been carried out within an ethical framework, e.g. if the severity of the experimental procedure is not justified by the value of the knowledge gained.

Guidelines for Authors

1. COVER LETTER

The initial submission must include a cover letter. This should be addressed to the Editor in Chief, Professor Lygidakis. This should explain any deviations from the standard format and any potential conflicts concerning the exclusive nature of the paper. The author is welcome to supply a brief description of the paper together with 3-5 names, addresses, phone numbers and e-mail addresses of potential reviewers. The names and complete mailing addresses (including e-mail addresses) of all co-authors should be included in the cover letter.

2. PAPER LAYOUT

Depending on the type of article it should contain following sections:

a. Original Paper:

- Abstract

- Introduction

- Methodology

- Results

- Discussion

- Conclusions (Optional)

- Acknowledgements (Optional)

- References

b. Review:

- Summary

- Introduction

- Ordered process according to authors style

- Discussion

- Conclusions (Optional)

- Acknowledgements (Optional)

- References

b. Surgical Techniques:

- Summary

- Introduction

- Surgical technique

- Discussion

- Conclusions (Optional)

- Acknowledgements (Optional)

- References

3. ABSTRACT

Abstract must be no longer than 200 words. Do not include abbreviations, references or footnotes in the abstract. The abstract must have four sections:

• **Background/Aims**, outlining the precise purpose of the paper

• **Methodology**, giving a brief description of materials used and a concise explanation of the methods

• **Results**, providing a summary of the findings

• **Conclusions**, explaining the significance of the work.

4. SECTIONS:

a. INTRODUCTION
The introduction should give brief background information and state the reasons and purposes behind the study.

b. METHODOLOGY
This section must give sufficient information to permit detailed evaluation and duplication of the work by other investigators. Ethical guidelines followed must be described. Approval of institutional human research review committees or animal welfare committees should be cited. An outline of the statistical methods should be included here.

c. RESULTS
In this section, the findings of the work should be presented. Use tables and figures where appropriate to improve the clarity of the presentation.

d. DISCUSSION
Discuss the results in relation to other published works in the same field. Offer explanations for any differences between the presented work and previous studies. Identify hypotheses and speculation clearly.

e. ACKNOWLEDGEMENTS

5. LANGUAGE

The standard language of the journal is American English. **Any manuscripts which are considered by discretion of the Editor as requiring language correction will not be prepared for publication until professional language correction has been performed**.

6. ABBREVIATIONS

The first appearance in the text should be written in full, with the abbreviation given afterwards in parentheses. Subsequently, only the abbreviation should be given. See below for common abbreviations.

7. NUMBERS

A number at the beginning of a sentence should be written in full. Otherwise, a number should be given in digit form.
Example: The control group included 60 subjects (34 men and 26 women). Forty-five of them were healthy volunteers (24 men and 21 women).
There should not be a space between numbers and mathematical symbols or measurement values.
Example: 35%; $p<0.021$; 1.5cm; $2560\times103/\mu L$

8. REFERENCES

Number references consecutively in the order in which they appear in the text. Punctuation, order, capitalization and spacing must conform **EXACTLY** like the example given below.

1) **Primrose JN, Axon ATR, Johnston D, et al:** Highly selective vagotomy and duodenal ulcers that fail to respond to H2 receptor agonists. Hokkaido Igaku Zasshi 1988; 296:1031-1045. (In Japanese with English abstract)

NOTE:

• No period (full-stop) after reference number

• No periods between or after initials

• Colon after final name

• Only first letter of title capitalized

• Period at end of title

• Journal abbreviation conforming to Index Medicus, National Library of Medicine (see below) semi-colon after year

• Colon, no space between issue number and page numbers

• Hyphen, no spaces between page numbers

• Period at end

9. TABLES

Each tables should be in a separate Word file **(please type into a WORD document TABLE, do not insert the table as a picture)**. Place the table number and title above the table, and any explanatory notes below the table. Please do not use tabs or spaces to separate columns, but create a TABLE in Word.

10. FIGURE LEGENDS

Figure Legends should be typed in the main text file. Figures should be numbered in order of presentation in the text. The legends should permit interpretation of the figures without reference to the text.

11. FIGURES

Use only the minimum number of figures required to illustrate a point. Figures should be submitted with jpeg/jpg or tiff format only, high resolution at least 300 dpi.

PRINTED VERSION GALLEY PROOFS

As soon as a manuscript has been edited and typeset ready for final printing, **a copy of the galley proof will be sent to the author by e-mail as a PDF file.** It is therefore very important to keep the online

system informed of current e-mail addresses and correspondence details. Timely correction of the galley proof is essential to ensure speedy publication of the article. At this stage, authors may make only minor corrections; major rewrites cannot be accepted, nor can the author make significant changes to the manuscript (i.e. add new data, figures, etc.). At this point, the author may order reprints. As the journal tries to minimize the cost of publication to allow for a wider distribution, the journal must charge for reprints. The cost is based on the number of printed pages in each article. The minimum order is 100 reprints.

COMMON ABBREVIATIONS

ADP adenosine diphosphate

ADPase adenosine diphosphatase

ALT alanine aminotransferase

anti-HAV antibody to hepatitis A virus

anti-HBc antibody to hepatitis B core antigen

anti-Hbe antibody to hepatitis B e antigen

anti-HBs antibody to hepatitis B surface antigen

anti-HCV antibody to hepatitis C virus

anti-HDV antibody to hepatitis D (delta) virus

AST aspartate aminotransferase

ATP adenosine triphosphate

ATPase adenosine triphosphatase

BUN blood urea nitrogen

CAH chronic active hepatitis

CD Crohn’s Disease

CDAI Crohn’s Disease Activity Index

HAV hepatitis A virus

HBcAg hepatitis B core antigen

HBeAg hepatitis B e antigen

HBsAg hepatitis B surface antigen

HBV hepatitis B virus

HCC hepatocellular carcinoma

HCV hepatitis C virus

HDV hepatitis D (delta) virus

H&E hematoxylin and eosin stain

IBD inflammatory bowel disease

Ig immunoglobulin

KICG plasma disappearance rate of indocyanine green

NSAID non-steroidal anti-inflammatory drug

PBS phosphate-buffered saline

RBC red blood cell

RIA radioimmunoassay

SGOT serum glutamic oxaloacetic transaminase

SGPT serum glutamic pyruvic transaminase

SI saturation index

TPN total parenteral index

UC ulcerative colitis

UNITS

°C degree(s) Celsius

cpm counts per minute

cps counts per second

cm3 cubic centimeter(s)

cycle/min cycles per minute

cycle/s cycles per second

day(s) day(s)

d density

°F degree(s) Fahrenheit

g gram(s)

g gravity, gravities

t½ half-life

Hz hertz

h hour(s)

in inch(es)

IU international unit(s)

K kelvin

kcal kilocalorie(s)

kg kilogram(s)

L liter(s)

mL milliliter(s)

µL microliter(s)

m meter(s)

µm micrometers

x magnification

mile/h mile(s) per hour

mm Hg millimeters of mercury

min minute(s)

Mol/L molar

mo month(s)

mol mole(s)

N normal

oz ounce(s)

lb pound(s)

rpm revolutions per minute

rps revolutions per second

s second(s)

U unit(s)

V volt(s)

W watt(s)

wk week(s)

yr year(s)

STATISTICAL TERMS

x2 method chi-squared method

r correlation co-efficient

df degrees of freedom

x mean

NS not significant

n number of observations

p probability

SD standard deviation

SE standard error

SEM standard error of the mean

F variance ratio