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***COLHEITA E ESTUDO DOS NÓDULOS
CARDIONECTORES:
UM CONTRIBUTO PARA O ESCLARECIMENTO
DE MORTES DE CAUSA DESCONHECIDA
ARTIGO CIENTÍFICO***

ÁREA CIENTÍFICA DE MEDICINA LEGAL

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Índice

1. INTRODUÇÃO.....	1
2. MATERIAL E MÉTODOS.....	3
3. RESULTADOS	5
4. DISCUSSÃO.....	17
5. CONCLUSÕES.....	20
6. AGRADECIMENTOS	21
7. REFERÊNCIAS BIBLIOGRÁFICAS	22
8. ANEXOS.....	24

Índice de Figuras

Fig. 1: Distribuição por género/grupo etário da amostra total do sistema cardionector estudada, referente a casos de morte de causa desconhecida	5
Fig. 2: Distribuição por género da amostra total do sistema cardionector estudada, referente a casos de morte de causa desconhecida	5
Fig. 3: Distribuição por causa de morte da amostra total do sistema cardionector estudada, referente a casos de morte de causa desconhecida	6
Fig. 4: Distribuição das alterações do sistema cardionector face à patologia cardíaca e não cardíaca verificada, referente a coorte da amostra estudada.....	7
Fig. 5: Distribuição das alterações do sistema cardionector face à patologia cardíaca, referente a coorte da amostra estudada	7
Fig. 6: Distribuição das alterações do sistema cardionector face à patologia cardíaca e ao género, referente a coorte da amostra estudada	8
Fig. 7: Distribuição por grupo etário de coorte da amostra do sistema cardionector estudada, referente a casos de morte por patologia cardíaca.....	8
Fig. 8: Distribuição por género das mortes cardíacas isquémicas e não isquémicas, de coorte da amostra estudada	9
Fig. 9: Distribuição total do tipo de alterações do sistema cardionector face à patologia cardíaca isquémica e não isquémica, referente a coorte da amostra estudada	10
Fig. 10: Distribuição do tipo de alterações do sistema cardionector face ao género, referente a coorte da amostra estudada.....	10
Fig. 11 a Fig. 19: Imagens histológicas	11-16

RESUMO: Colheita e estudo dos nódulos cardionectores:

um contributo para o esclarecimento de mortes de causa desconhecida

Introdução: Num Serviço de Patologia Forense a diminuição de mortes de causa indeterminada, que não devido à existência de putrefacção, constitui sempre um objectivo a alcançar, quer a nível internacional quer nacional. A maioria destes casos deve-se a situações de morte natural em que não foi possível, apesar da realização de exames complementares, determinar concretamente qual a patologia orgânica que desencadeou a morte.

Atendendo a que a morte natural de causa cardíaca é aquela que mais frequentemente se encontra em patologia forense, particular atenção deve ser dedicada a este órgão, nomeadamente no que respeita ao sistema de condução. De facto, embora uma arritmia não se documente num exame *post mortem*, o estudo do sistema de condução pode revelar alterações que sugiram e/ou permitam o diagnóstico do evento fatal.

Objectivos: O objectivo deste estudo consiste em contribuir para ao esclarecimento de causa de mortes desconhecidas através da análise do sistema cardionector em autópsias da Delegação do Centro do INML, IP.

Material e Métodos: Para tal, nos últimos anos tem-se procedido a um estudo prospectivo relativamente ao sistema de condução cardíaco em casos com suspeita de morte natural, pertencentes a indivíduos com idade superior a 18 anos, em que não foi possível, no decorrer da autópsia forense, determinar a causa efectiva da morte. Foram colhidas cem amostras de nódulos sinusal e atrioventricular, que se prepararam segundo a técnica de rotina, complementada com a coloração especial "Tricrómio de Masson" (de fácil execução e não onerosa).

Resultados: Verificou-se a presença de alterações do sistema cardionector em mais de um terço dos casos de morte por patologia cardíaca, as quais ou permitiram esclarecer a

causa de morte ou, embora não tendo contribuído directamente para a morte, devem ser valorizadas.

Conclusões: Este trabalho mostra que o estudo do sistema cardionector permite, com exigências mínimas - quer económicas quer de trabalho - em casos seleccionados, realizado por profissionais experientes, propor um diagnóstico da causa de morte, reduzindo assim, o número de mortes de causa desconhecida.

Palavras-Chave: nódulos cardionectores; diagnóstico; morte desconhecida

Evaluation of the cardiac conduction system: a contribution to the elucidation of unknown cause of death

Introduction: In a national or international Forensic Pathology Department, the decrease of unknown cause of death not owing to putrefaction, is always a goal to achieve. The majority of these cases are due to a natural cause of death, whose organic pathology that triggered death was not possible to determine, despite accurate studies.

Given the fact that cardiac pathology is the most frequent cause of natural death in forensic pathology, special attention should be devoted to the heart, in particular to the conduction system. In fact, although an arrhythmia is not documented in a *post mortem* examination, the study of cardiac conduction system may reveal abnormalities that can suggest and/or allow the diagnosis of the fatal event.

Objective: The aim of this study is to contribute to the clarification of unknown cause of death through the examination of the cardiac conduction system in autopsies performed at the Central Branch of the National Institute of Legal Medicine.

Material and Methods: To achieve this purpose, in the past recent years, a prospective study of the cardiac conduction system in cases of suspected natural death from

individuals aged over 18 years old, in which it was not possible, during the *post mortem* exam, to determine a precise cause of death, has been carried out.

One hundred samples of atriosinusal and atrioventricular nodes were collected, prepared according to the routine technique, complemented with the special staining "Masson Trichrome" (technically of easy procedure and not expensive).

In more than one third of the death cases due to cardiac pathology, morphologic alterations of the cardiac conduction system were observed and either allowed the clarify the fatal event or – although not directly related to the death – must be reported and contextualized.

Conclusions: The study of the cardiac conduction system allows, with minimal economic and laborious requirements, in selected cases, performed by trained and experienced professionals, to reach the cause of death, thus reducing, the number of “deaths of unknown cause”.

Passwords: cardiac conduction system; diagnosis; unknown cause of death

1. Introdução

Segundo a *Organização Mundial de Saúde*, em 2008, 49,7% da mortalidade na Europa foi devida a patologia cardiovascular. Tal situação é também observada na prática forense. De facto, o Instituto Nacional de Medicina Legal realiza um número significativo de autópsias por mortes de causas naturais, nomeadamente devidas a patologia cardiovascular.

No entanto, apesar de investigação exaustiva, nem sempre é possível diagnosticar a situação patológica que condicionou a morte. É neste contexto que recomendações propostas pela *Association for the European Cardiovascular Pathology* se revestem de particular importância na investigação *post mortem*, especialmente em casos de morte – súbita ou não - suspeita de causa cardíaca (Basso C et al, 2008). Segundo este protocolo, um dos constituintes cardíacos cuja análise em casos seleccionados se afigura mandatária, é o sistema cardionector, uma vez que este é o responsável pela iniciação e coordenação dos batimentos cardíacos.

No ano de 1852 foi demonstrado por Stannius que o tecido muscular cardíaco especializado era o responsável pela iniciação e difusão dos impulsos eléctricos cardíacos (Sánchez-Quintana D, 2003). Estudos subsequentes permitiram tanto a sua descrição anatómica e histológica, como a caracterização daquelas fibras musculares cardíacas especiais. Em 1906, Tawara confirmou a existência de um ramo muscular, já descrito por His em 1893. Keith e Flack corroboraram a existência do sistema de Tawara. Um ano depois descreveram a estrutura do nódulo átrio-sinusal (Waller, 1993, Sánchez-Quintana, 2003).

Embora, sob o ponto de vista clínico, seja mais fácil a constatação de perturbações do ritmo cardíaco, já no exame *post mortem* tal é praticamente impossível de demonstrar objectivamente. O estudo do sistema cardionector - mórmente dos

nódulos átrio-sinusal, atrioventricular, do feixe de His e respectivos ramos - é obrigatório em casos de morte súbita, inexplicada, não violenta, sem lesões aparentes ou naquelas em que existe uma informação clínica prévia de perturbações do ritmo cardíaco. Quaisquer alterações documentadas no Sistema de Condução Cardíaco podem ser relevantes e até permitir o diagnóstico da causa da morte.

Este trabalho foi idealizado com o intuito de objectivar a contribuição real do estudo do Sistema de Condução Cardíaco na determinação da causa de morte numa população adulta (1), bem como na redução do número de mortes de causa indeterminada (2).

2. Material e Métodos

Entre os anos de 2007 e 2012 (Janeiro), cem amostras do Sistema de Condução Cardíaco (23 do género feminino, 77 do masculino) procedentes de autópsias do Serviço de Patologia Forense da Delegação do Centro do Instituto Nacional de Medicina Legal, IP, foram colhidas pela autora, de acordo com as técnicas descritas por Ludwig, 2002, Gulino, 2003, Sheaff e Hopster, 2005. Estas amostras pertenciam a cadáveres sem indícios de putrefacção, referentes a indivíduos com mais de 18 anos, vítimas de morte inesperada, não violenta e de causa desconhecida. Nestes casos, a verificação da morte ocorreu à entrada de um Serviço de Saúde ou foi constatada na residência, rua ou local de trabalho. Em todos os casos, os resultados toxicológicos foram considerados irrelevantes.

Todos os órgãos foram avaliados macroscopicamente, tendo sido colhidas amostras - tanto as convencionais como de áreas patológicas - para exame microscópico.

Em dois terços dos casos, o Sistema de Condução Cardíaco foi recolhido sem fixação anterior do coração. No outro terço, o coração foi previamente fixado em formol a 10%. Após a fixação, os segmentos atriosinusal e atrioventricular foram colhidos. Cada um dos segmentos foi seccionado segundo o maior eixo em 6-10 blocos, cada um dos quais com 2-3 milímetros de espessura. Cada bloco foi posteriormente seccionado, de forma seriada, tendo cada corte 3 μ de espessura. As lâminas foram coradas com Hematoxilina/Eosina (HE) e tricrómio de Masson (TM). Em casos seleccionados, a coloração de elastic van Gieson (EvG) foi também utilizada.

Todas as lâminas foram revistas pela mesma Anátomo-Patologista. Os resultados dessa revisão são os aqui apresentados.

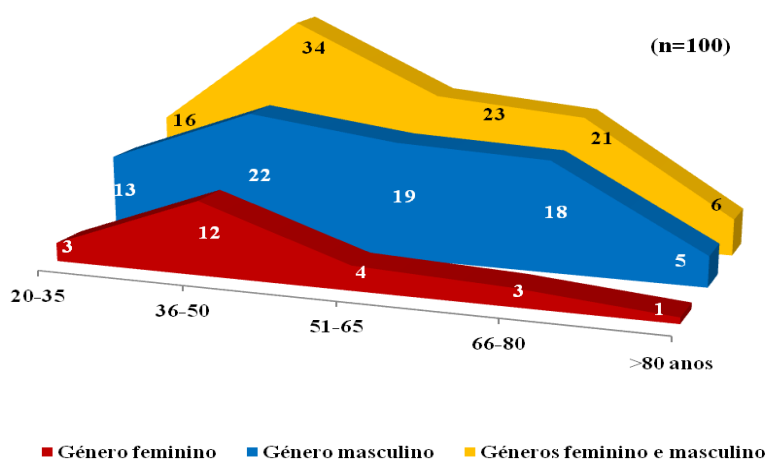
Aquando da análise de cada componente do Sistema de Condução Cardíaca (SCC), os autores: 1) Procuraram a “presença” ou “ausência” de alterações da cito-arquitectura normal. 2) Se presentes, caracterizaram-nas e/ou graduaram-nas. 3) A quantificação foi semi-quantitativa (ligeira = 0-25% da área do componente do SCC em observação, moderada = 25-50%, marcada = 50-100%)

Para obtenção e exportação de dados foi utilizado o ficheiro *Tanato*, elaborado desde 2003 pela autora, pertencente ao programa *FileMaker Pro 6, 2002L*; e utilizado no âmbito do Serviço de Patologia Forense da Delegação do Centro do INML, IP desde 2003. Recorreu-se ao programa *Microsoft Office Excel, 2007* para análise estatística e representação dos dados.

3. Resultados

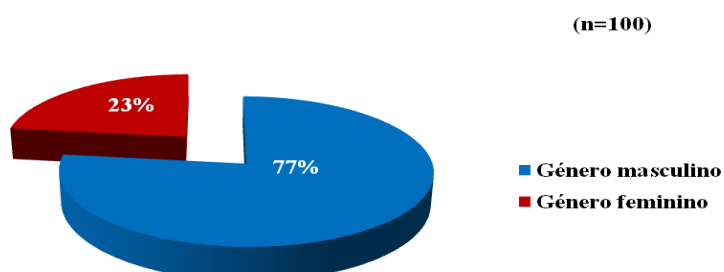
Os cem casos estudados pertenceram a uma amostra populacional adulta, com idades entre os 20 e os 87 anos, sendo a idade média igual a 51.9 anos, a moda igual a 53 anos e a mediana igual a 49 anos (Fig. 1).

Fig. 1: Distribuição por género/grupo etário da amostra total do sistema cardionector estudada, referente a casos de morte de causa desconhecida



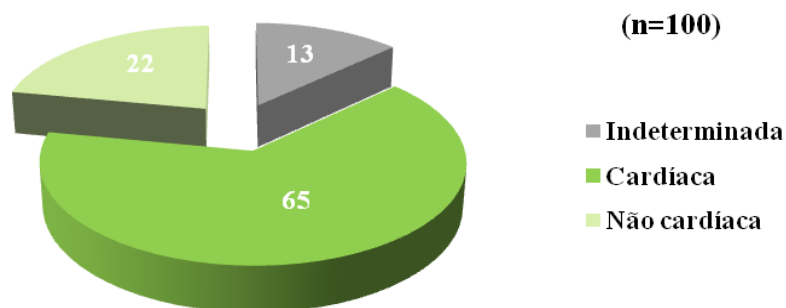
O maior número de casos ocorreu em indivíduos do género masculino (Fig. 2).

Fig. 2: Distribuição por género da amostra total do sistema cardionector estudada, referente a casos de morte de causa desconhecida



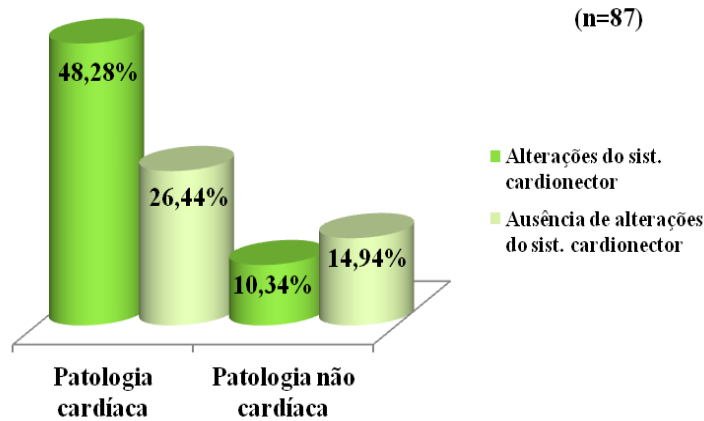
Constatou-se que a patologia cardiovascular era a causa mais frequente de morte (Fig. 3).

Fig. 3: Distribuição por causa de morte da amostra total do sistema cardionector estudada, referente a casos de morte de causa desconhecida.



Tanto as mortes de causa cardíaca como não cardíaca (n=87) revelaram alterações do Sistema de Condução Cardíaco, quer isoladamente quer em associação e presentes na periferia ou no próprio sistema cardionector. Estas alterações incluíram *fibrose, lipomatose, calcificações, alterações vasculares, outras* (hipertrofia, dilatação, atrofia das fibras musculares cardíacas e presença de células inflamatórias). Apenas 10,34% destas alterações foram observadas em casos de morte não cardíaca contra 48,28 da cardíaca (Fig 4). Nas mortes indeterminadas apenas 2 casos apresentavam alterações inespecíficas.

Fig. 4: Distribuição das alterações do sistema cardionector face à patologia cardíaca e não cardíaca verificada, referente a coorte da amostra estudada



Quando se consideraram apenas as mortes de causa cardíaca, as alterações do Sistema de Condução Cardíaco estavam presentes em 42 casos, representando 64,62% (52,31% no género masculino) (Fig. 5, 6).

Fig. 5: Distribuição das alterações do sistema cardionector face à patologia cardíaca, referente a coorte da amostra estudada

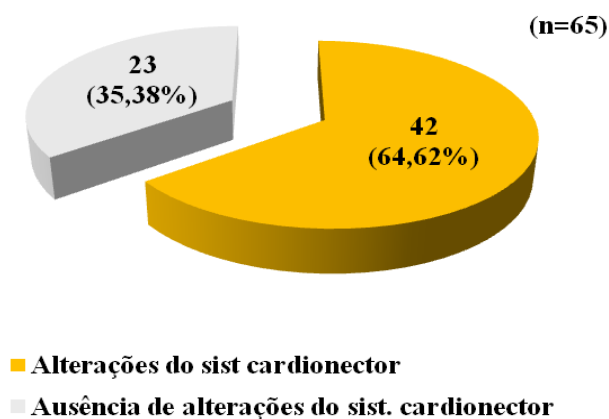
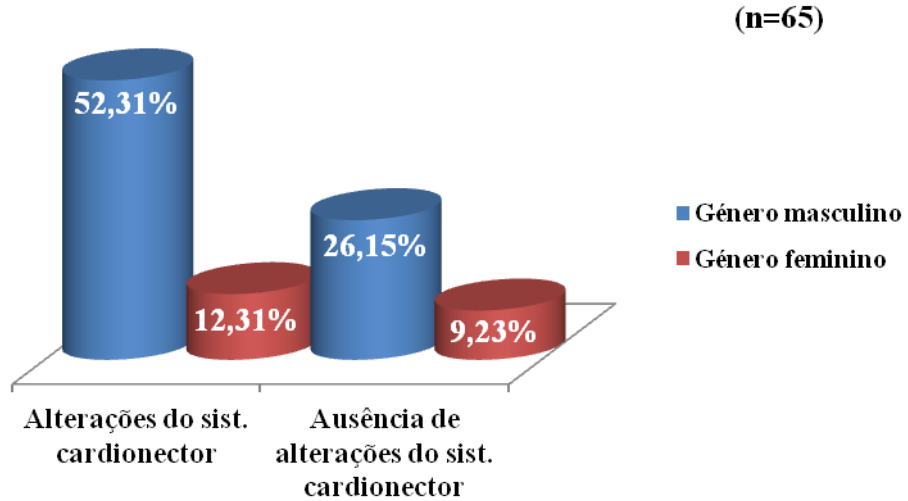
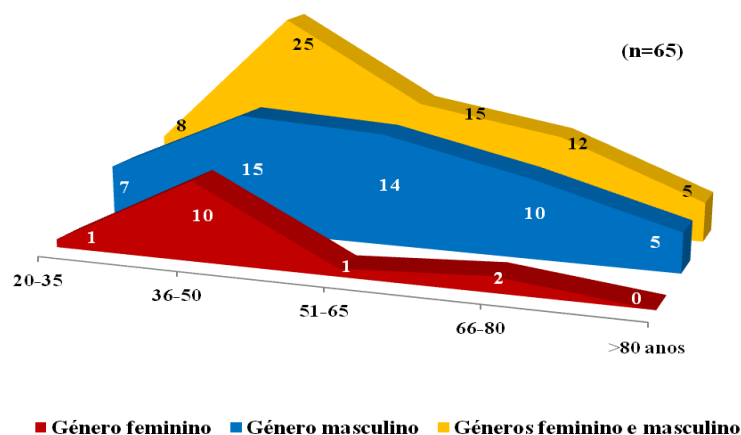


Fig. 6: Distribuição das alterações do sistema cardionector face à patologia cardíaca e ao género, referente a coorte da amostra estudada



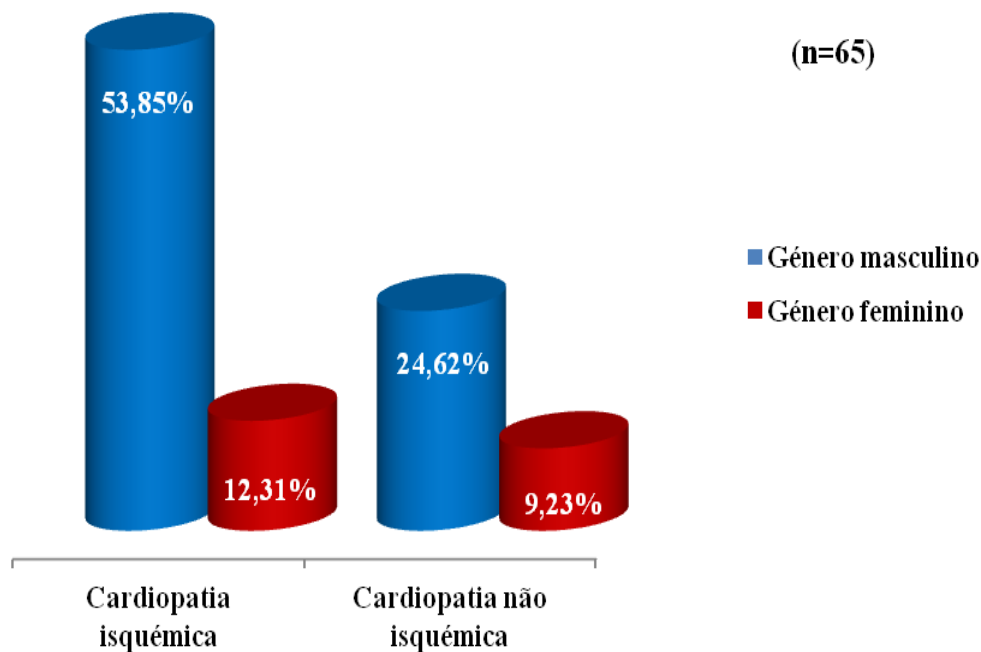
O género masculino foi predominante (n=49), com idades compreendidas entre os 20 e os 87 anos, enquanto a idade no género feminino se encontrava entre os 24 e os 85 anos (Fig. 7).

Fig. 7: Distribuição por grupo etário de coorte da amostra do sistema cardionector estudada, referente a casos de morte por patologia cardíaca



Subdividiu-se a patologia cardíaca encontrada, em isquémica e não isquémica. Em cada subgrupo também o género masculino foi preponderante (Fig. 8)

Fig. 8: Distribuição por género das mortes cardíacas isquémicas e não isquémicas, de coorte da amostra estudada



Nas mortes de causa cardíaca, as alterações do Sistema de Condução Cardíaco contabilizaram um total de 64, quando contadas *per se* e não em associação (26 fibrose, 20 lipomatose, 5 calcificações, 4 alterações vasculares e 9 outras alterações). A distribuição por género, tipo de patologia cardíaca e alterações do SCC são representadas nas Fig(s) 9 e 10.

Fig. 9: Distribuição total do tipo de alterações do sistema cardionector face à patologia cardíaca isquémica e não isquémica, referente a coorte da amostra estudada

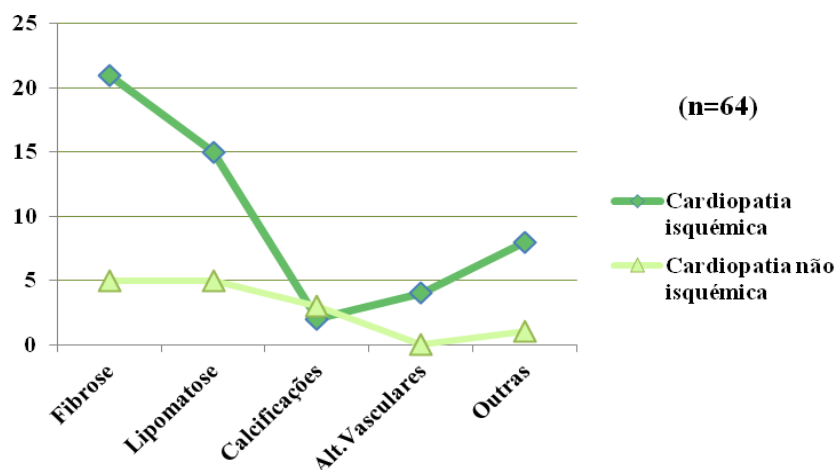
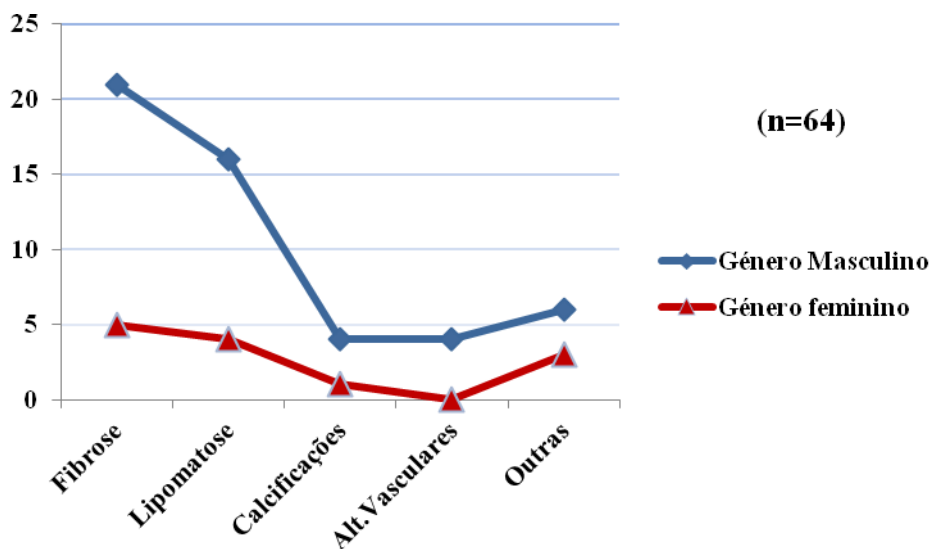


Fig. 10: Distribuição do tipo de alterações do sistema cardionector face ao género, referente a coorte da amostra estudada



Nos 42 casos de alterações do sistema cardionector, para além das alterações isoladas, verificou-se, mais frequentemente, a associação de fibrose com lipomatose

(14,29%), fibrose com outros tipos de alterações (14,29%) e lipomatose com outros tipos de alterações (7,1%).

A fibrose, isolada ou associada, manifestou-se a partir dos 50 anos em 76,9%, sendo mais acentuada no género masculino (21 casos). Nas restantes alterações do sistema cardionector não foi possível estabelecer um padrão face às idades.

Em relação às alterações denominadas como “Outras”, os autores enfatizam a presença de atrofia do nódulo atrio-sinusal, pertencente a indivíduo do género masculino, com idade de 87 anos.

Em 9 dos 42 casos, as alterações do SCC foram reconhecidas como sendo capazes de influenciar o desfecho fatal. De referir que todos os cinco casos de calcificação do Sistema de Condução Cardíaco (4 do género masculino e 1 do feminino) com excepção de um, se encontravam na série destes nove casos, pertencendo a indivíduos com idades entre os 50 e os 60 anos e um com idade superior a 80 anos.

Fig. 11: Indivíduo do género masculino, com 49 anos, com causa de morte cardíaca não isquémica. A imagem histológica apresenta calcificações que comprimem o feixe de His

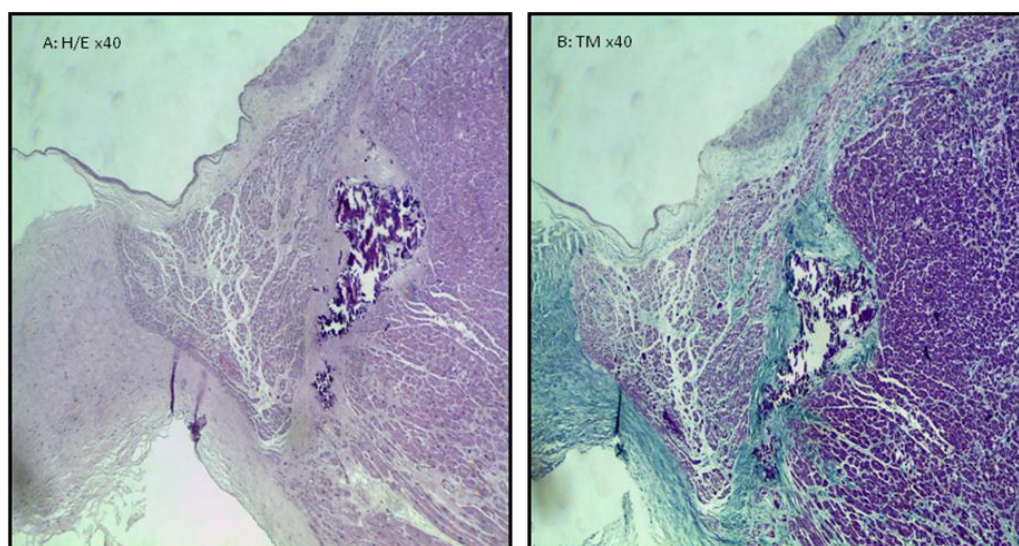


Fig. 12: Indivíduo do género masculino, com 50 anos, com causa de morte cardíaca não isquémica. A imagem histológica apresenta calcificações compressivas e fibrose ligeira no feixe de His e ramo

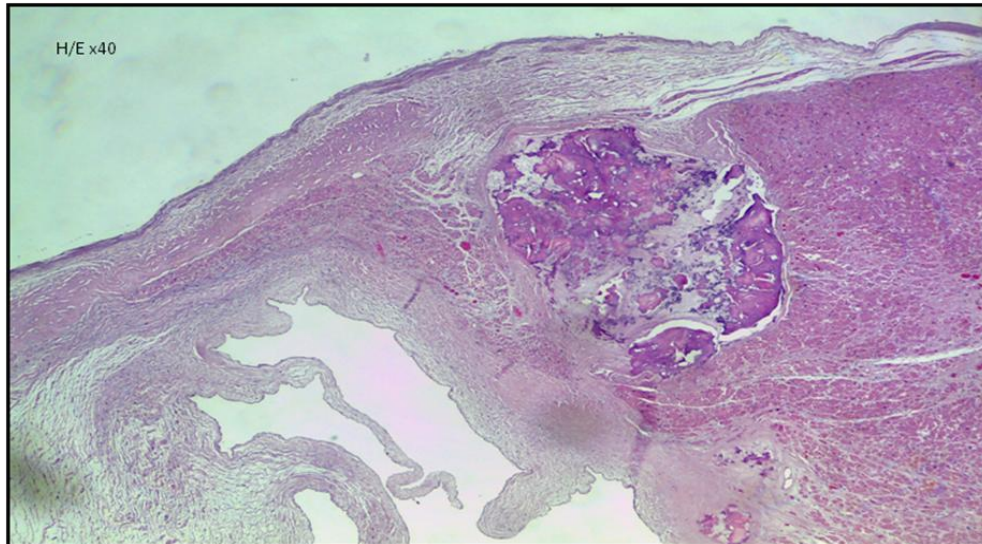
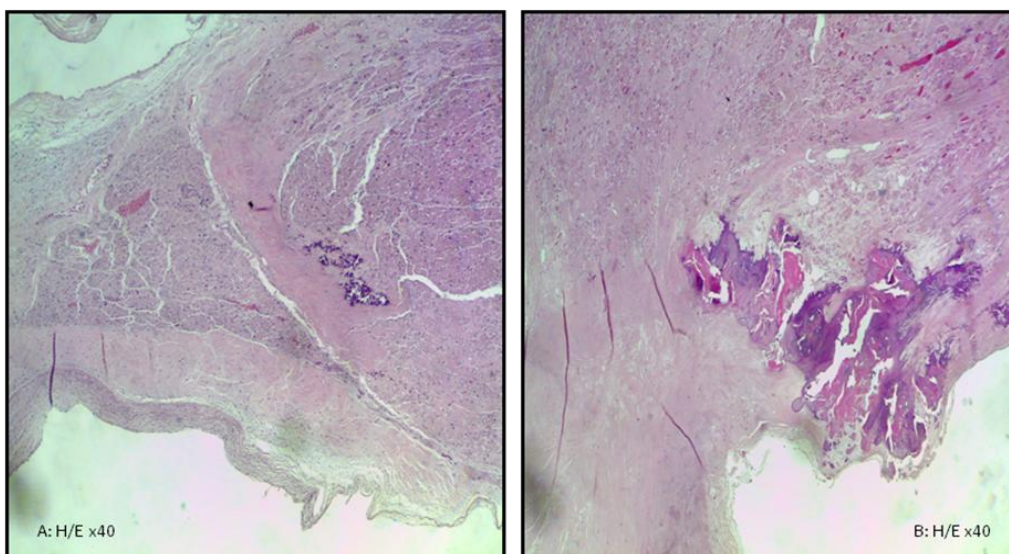


Fig. 13: Indivíduo do género masculino, com 85 anos, com causa de morte cardíaca não isquémica e antecedentes de diabetes mellitus 2 e hipertensão arterial. As imagens histológicas apresentam em A e B calcificações compressivas do feixe de His e em C fibrose ligeira (0-25%) também do feixe de His



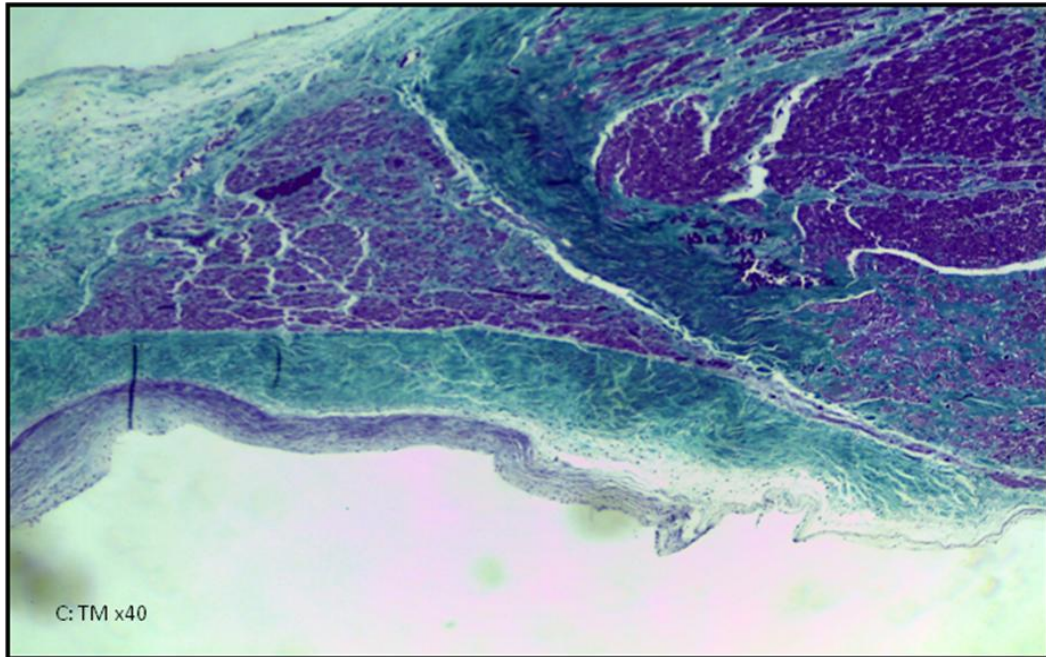


Fig. 14: Indivíduo do gênero masculino, com 45 anos e causa de morte cardíaca não isquêmica. A imagem histológica apresenta lipomatose marcada (50-100%) no nódulo atrioventricular

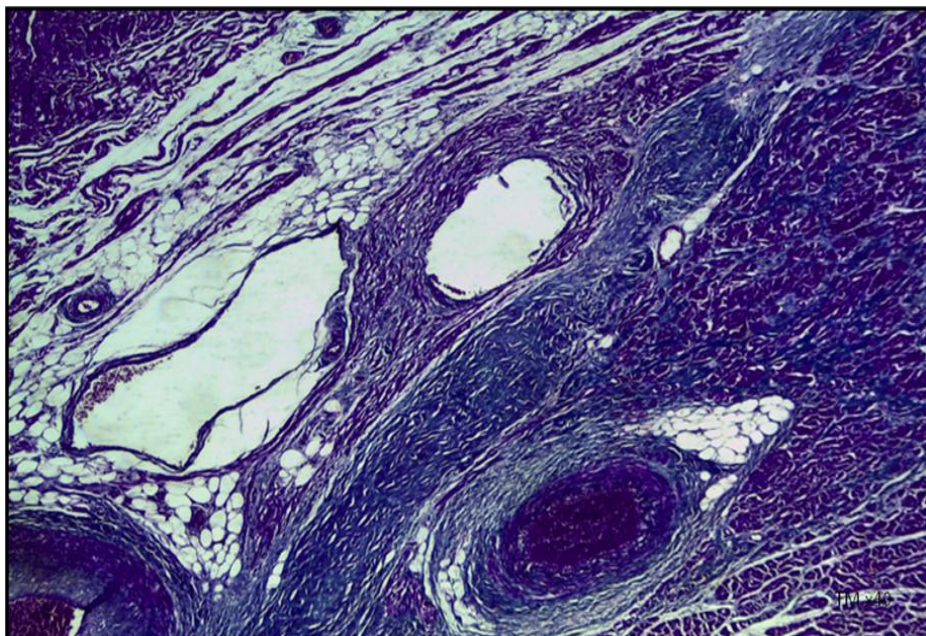


Fig. 15: Indivíduo do género feminino, com 74 anos e causa de morte cardíaca isquémica. A imagem histológica apresenta lipomatose marcada do nódulo atrioventricular (50-100%)

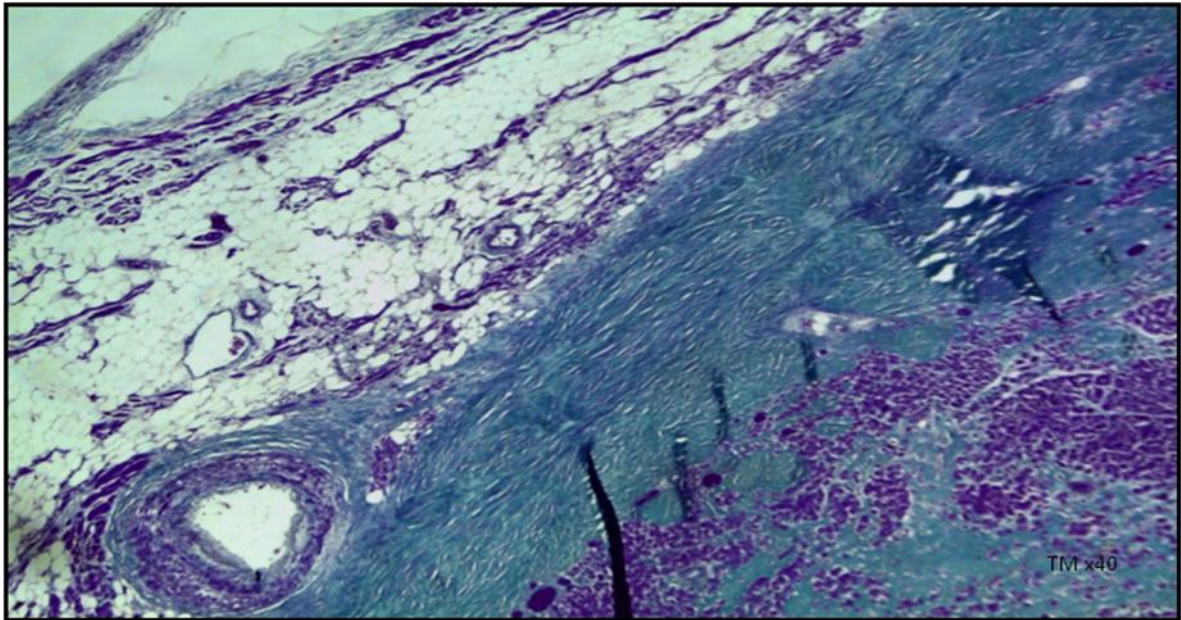


Fig. 16: Indivíduo do género masculino, com 67 anos e causa de morte cardíaca isquémica. A imagem histológica apresenta lipomatose marcada no feixe de His (50-100%)

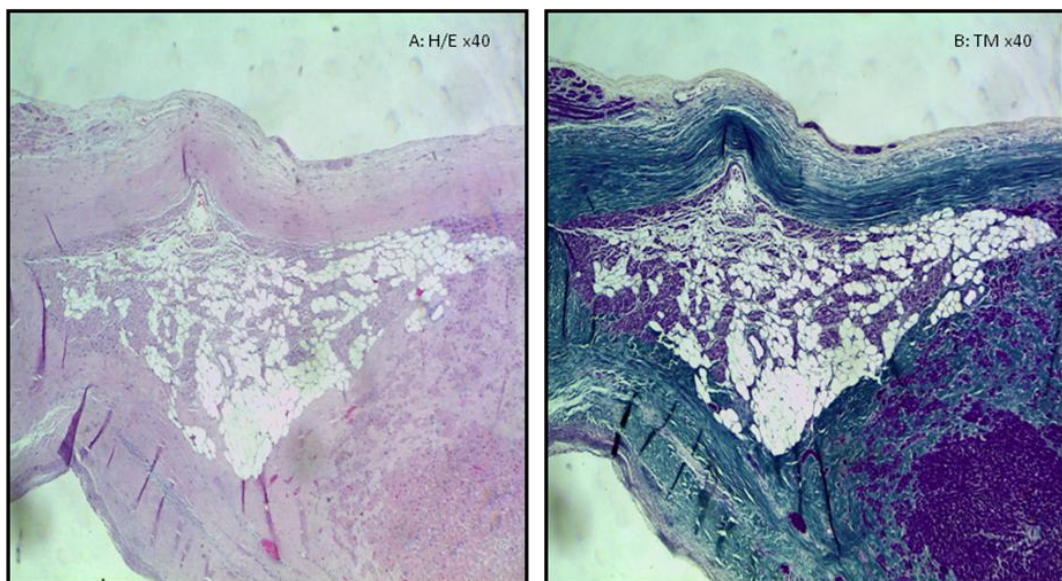


Fig. 17: Indivíduo do género feminino, com 64 anos e causa de morte cardíaca isquémica. A imagem histológica apresenta fibrose moderada (25-50%) no nódulo atrioventricular

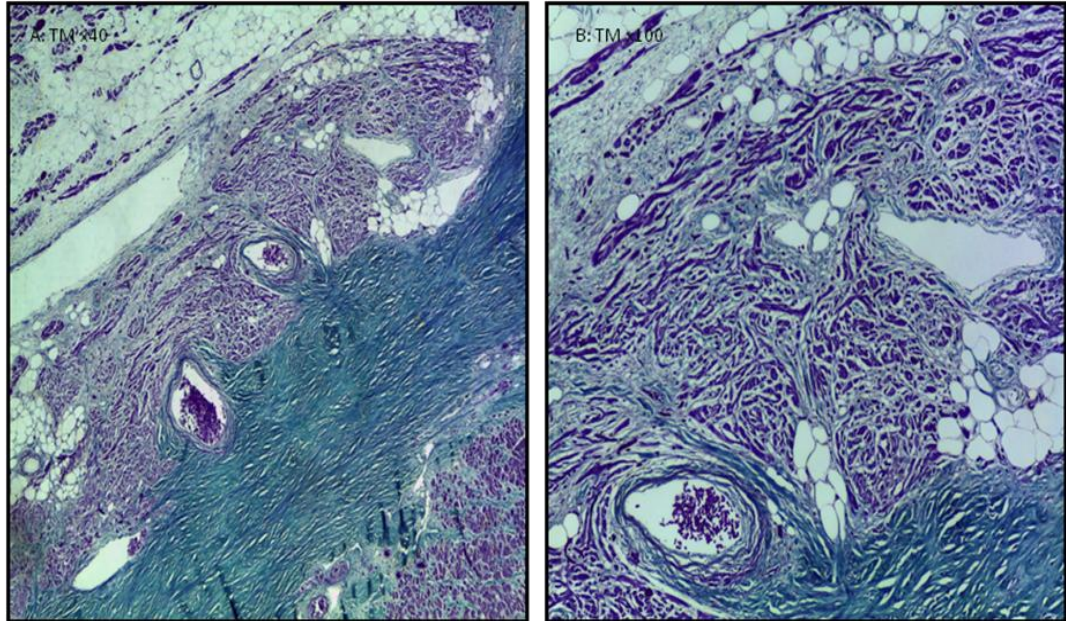
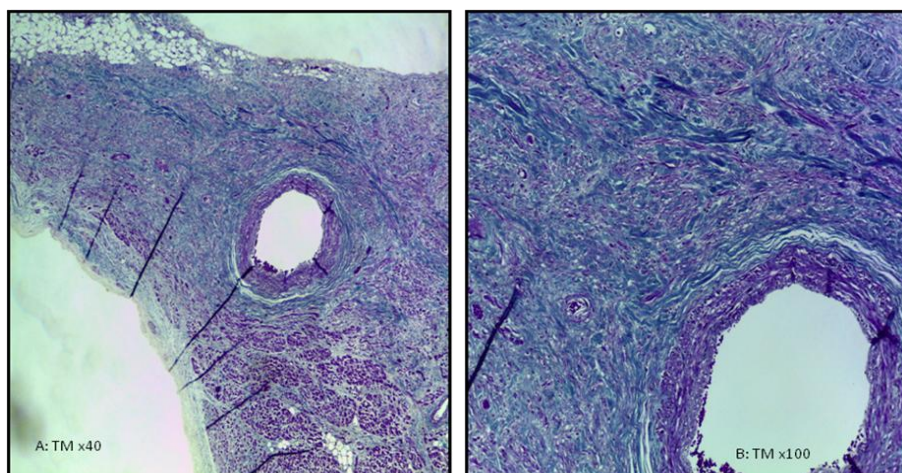


Fig. 18: Indivíduo do género masculino, com 85 anos, antecedentes de insuficiência renal crónica em hemodiálise e implantação de *pacemaker*, com causa de morte cardíaca isquémica. As imagens histológicas apresentam fibrose moderada (25-50%) e atrofia celular no nódulo átrio-sinusal (A e B) e fibrose moderada (25-50%) no nódulo atrioventricular (C)



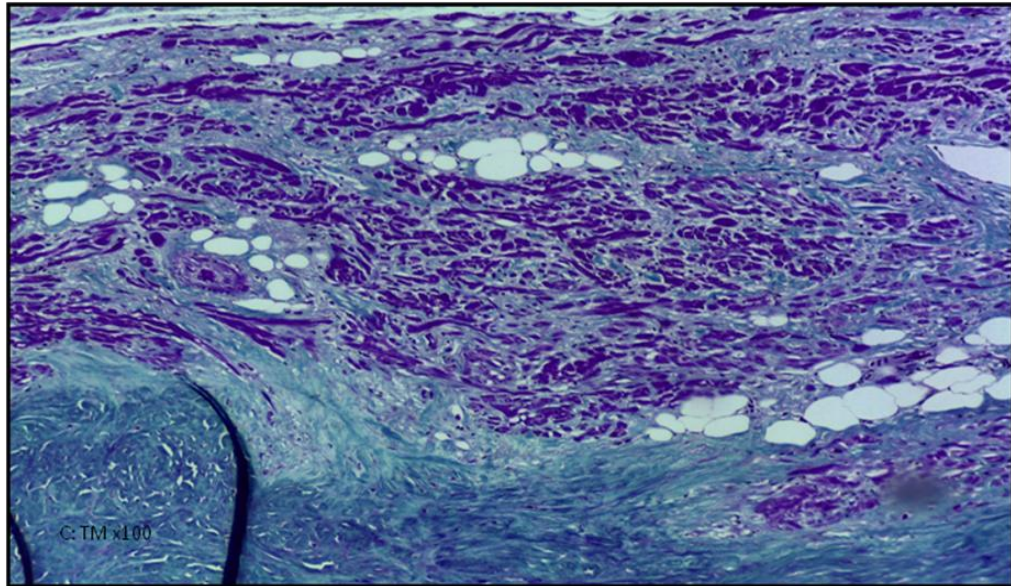
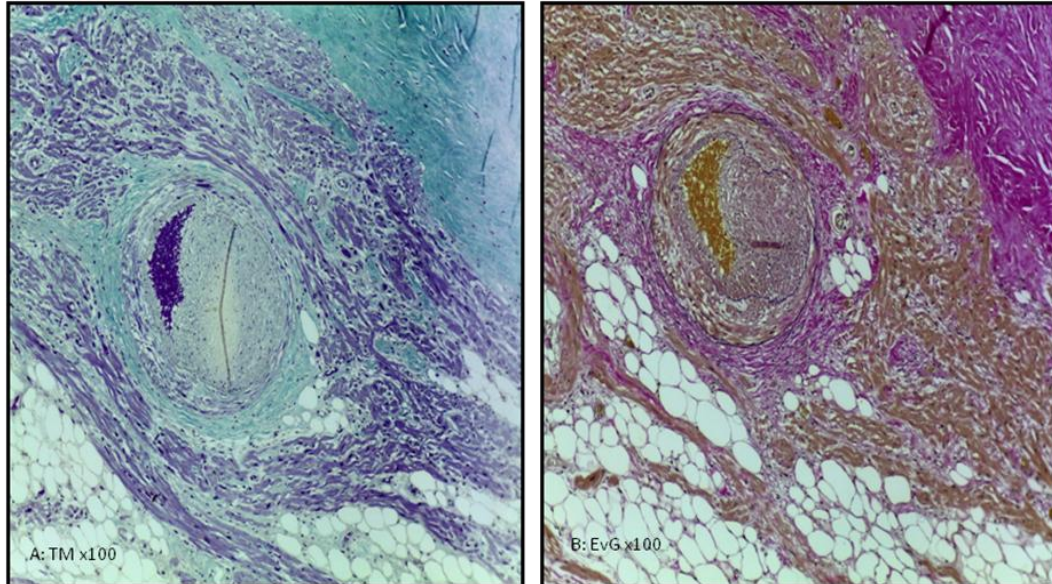


Fig. 19: Indivíduo do género masculino, com 45 anos e causa de morte cardíaca isquémica. A imagem histológica apresenta alterações vasculares (hiperplasia fibrointimal) no nóculo atrioventricular



4. Discussão

Embora estejam descritos diferentes tipos de procedimentos para a colheita dos nódulos cardionectores, neste estudo foi usado o indicado por Ludwig (2002), Gulino (2003), Sheaff e Hopster (2005). Michaud et al (2002) haviam apresentado um método de colheita, considerado por estes autores como de mais fácil execução. Todavia, comparando os dois métodos, verificou-se que, enquanto um utilizava a metade direita do coração, o segundo recorria à metade esquerda, mais propriamente à área das valvas sigmóides. Após experimentação, não foi por nós reconhecida qualquer mais-valia neste método, podendo até eventuais alterações valvulares determinar colheitas menos precisas, motivo pelo que as amostras do presente estudo foram obtidas segundo a metodologia inicialmente referida.

À semelhança do referido por Ludwig (2002) e Sheaff e Hopster (2005), a colheita dos nódulos em corações previamente fixados em formol a 10% apresentou-se como vantajosa, já que melhora acentuadamente, quer a qualidade do corte dos segmentos, quer a conseqüente acuidade da avaliação microscópica pela Anátomo- Patologista.

Já anteriormente, trabalhos de Okada e Kawai (1983), Suarez-Mier et al (1995), Yixuan et al (2001), Cohle et al (2002), Michaud et al (2002) e Nishida et al (2002) haviam revelado alterações do sistema cardionector. No entanto, neles foram incluídos casos de morte não natural e/ou grupos etários muito jovens, pelo que não foi possível extrapolar a contribuição real deste sistema na morte de causa natural cardíaca no adulto. Por outro lado, a correlação entre a presença/quantificação das alterações e o evento fatal não se apresenta sistematicamente evidenciada.

O presente estudo mostra: 1) a existência de alterações no Sistema de Condução Cardíaco do adulto numa percentagem elevada de casos; 2) apesar das

diferenças tanto entre género como nos grupos etários, apenas uma percentagem muito reduzida de casos (9 em 100) foi considerada responsável ou contribuindo na realidade para a morte.

Quanto às alterações documentadas no Sistema de Condução Cardíaco, sabemos que:

A fibrose pode surgir com o envelhecimento/ mecanismos degenerativos, bem como por lesões isquémicas/hipóxicas. (Cotran et al, 1994)

A lipomatose pode ser a consequência de distúrbios metabólicos, lesões isquémicas/hipóxicas, envelhecimento/alterações degenerativas. (Cotran et al, 1994, Bharati S e Lev M, 2003)

As calcificações – tanto distróficas como metastáticas – podem ocorrer devido a lesão tecidual local ou por alterações do metabolismo/patologia renal. (Cotran et al, 1994).

A fibrose, a lipomatose e as calcificações são um obstáculo à iniciação e condução do impulso eléctrico; comprimem e/ou causam atrofia ou destruição das fibras especializadas musculares, sendo pois um potencial substracto para perturbações rítmicas (arritmias, bloqueios,...).

As alterações vasculares podem incluir a hiperplasia fibrointimal, a hipertrofia muscular da média, entre outras (Jing e Hu, 1997, Smith e Davis, 1997, Nishida et al. 2000). Estas alterações podem surgir em contextos variados, nomeadamente hipertensão, aterosclerose, envelhecimento, malformações arteriais. Os mecanismos isquémicos/hipóxicos podem levar directamente a arritmias ou indirectamente induzir as alterações do SCC previamente mencionadas.

O decorrer dos anos, associado à genética e ao estilo de vida, favorece o aparecimento das alterações já mencionadas. No entanto, os resultados obtidos neste

trabalho revelam que a mera presença das alterações do SCC podem não ser suficientes para causar a morte. A sua localização, distribuição, quantidade e eventual associação são da máxima importância.

Assim, o estudo do Sistema de Condução Cardíaco, em casos selecionados e realizado por profissionais experientes, pode de facto, indicar a causa de morte e reduzir o número de autópsias com causa de morte indeterminada.

5. Conclusões

1. O estudo do sistema cardionector, nomeadamente dos nódulos atriosinusal e atrioventricular, pode melhorar a qualidade do diagnóstico forense da causa de morte, em casos de autópsia “branca”.
2. Este estudo deve preferencialmente ser efectuado em casos de morte natural, em que, macroscopicamente não foi encontrada uma causa de morte com expressão morfológica, bem como naqueles em que é conhecida a existência de perturbações de ritmo.
3. A fixação do coração deverá ser efectuada, de preferência, antes da colheita/exame do sistema de condução cardíaco, por permitir melhor preparação e resultados microscópicos de maior acuidade.
4. A experiência na colheita e no estudo histológico do Sistema de Condução Cardíaco tem relevância na qualidade dos resultados obtidos.

6. Agradecimentos

A todos os elementos do Instituto Nacional de Medicina Legal, IP que apoiaram a mestranda na efectivação desta dissertação, com particular realce para os seus orientadores.

Uma palavra especial para a família, nomeadamente os seus Pais, que lhe demonstraram o valor da *palavra, trabalho e estudo*.

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8. Anexos

A mestranda anexa o Guia para Autores do *Journal of Forensic and Legal Medicine*, revista científica para a qual foi enviado o trabalho para publicação.



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AUTHOR INFORMATION PACK

TABLE OF CONTENTS

●	Description	p.1
●	Audience	p.1
●	Abstracting and Indexing	p.2
●	Editorial Board	p.2
●	Guide for Authors	p.4



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