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Suplemento –XLVIII Reunião Científica da Sociedade Anatómica Portuguesa/I Reunião Científica da Associação Anatómica Portuguesa.

EDITORIAL

EDIÇÃO CIENTÍFICA EM ANATOMIA O DILEMA DA INDEXAÇÃO E DO FACTOR DE IMPACTO

A realidade da edição científica passa pela exigência curricular dos autores da publicação em revistas indexadas e com factor de impacto. É de facto uma implacável evidência que condiciona o futuro das Revistas das Sociedades Científicas com pequeno número de associados, sobretudo quando os patrocínios, em época de crise económica mundial se tornam escassos.

Perante esta evidência, verifica-se uma concentração do fluxo de artigos que acedem à publicação nas poucas revistas indexadas na área médica em Portugal, que naturalmente aumentam as exigências de qualidade dos artigos candidatos à publicação nas mesmas.

Quando assumimos a coordenação da edição de *Archives of Anatomy*, partimos do pressuposto de termos, para além do corpo editorial médico, de uma equipa técnica que incluísse um tradutor, um *designer* e apoio informático específico para a revista, o que não aconteceu, pela escassez de meios financeiros. Procurámos uma solução que passou pela edição de um número de Anatomia, em parceria com a prestigiada Revista Acta Médica Portuguesa, de modo a que fosse publicado por ocasião do *XII Lisbon Joint Congress*, organizado em Junho de 2013 na Faculdade de Medicina da Universidade de Lisboa, sob a presidência do Professor Doutor António José Gonçalves Ferreira. A revista foi impressa em papel e constituiu um êxito a par do grande impacto que teve o referido evento.

Porém, o passo seguinte seria o da prossecução da mesma parceria, que não foi

possível manter nos termos iniciais pela alegada isenção que se pretendia manter face às solicitações de outras Sociedades Científicas Médicas.

Entretanto decorreram eleições na Associação Anatómica Portuguesa e a nova Direcção eleita para o biénio de 2014 – 2016 decidiu manter uma edição *online*, ficando sempre aberta e dialogante com todos os associados, de quem dependerá o futuro da publicação de *Archives of Anatomy*.

A existência de artigos arbitrados e de resumos das Comunicações apresentadas na XLVIII Reunião Científica da Sociedade Anatómica Portuguesa e I Reunião Científica da Associação Anatómica Portuguesa, permitem-nos a edição deste número de *Archives of Anatomy*, que incluirá uma pequena reportagem sobre o evento que teve lugar a 22 de Março de 2014, nas Instalações da Faculdade de Ciências Médicas da Universidade Nova de Lisboa, sob a presidência do Director do Departamento de Anatomia da mesma Faculdade, Professor Doutor João Goyri O'Neill. Haverá ainda uma apresentação do plano de acção para o biénio de 2014 – 2016, do presidente da Associação Anatómica Portuguesa, Professor Doutor Diogo Freitas Branco Pais.

O futuro da Revista será o que os Associados quiserem e estaremos sempre na postura de Bem-Servir a Causa da Anatomia e das Ciências Morfológicas em Portugal!

Ivo Álvares Furtado

(Editor-chefe de *Archives of Anatomy*)

EDITORIAL

SCIENTIFIC EDITION ON ANATOMY THE DILEMMA OF THE INDEX AND IMPACT FACTOR

The reality of scientific issue involves the curricular requirement of the authors to publish in indexed journals with impact factor. It is indeed a relentless evidence that determines the future of the scientific societies journals with a small number of members, especially when the sponsorships, in times of global economic crisis become scarce.

Given this evidence, there is a flux concentration of accessing the articles candidate to publish in the few indexed journals in medical field in Portugal, which naturally increase the quality requirements of the same articles.

When we took over the coordination of the edition of the Archives of Anatomy, we assumed terms, beyond medical editorial board, a technical team that included a translator, designer and dedicated IT support for the magazine, which did not happen, by the scarcity of financial support. We thought a solution that has passed through a number of editing Anatomy, in partnership with the prestigious journal *Acta Médica Portuguesa*, so that was published on the occasion of the XII Lisbon Joint Congress, organized in June 2013 at the Faculty of Medicine, University of Lisbon under the chairmanship of Professor José António Gonçalves Ferreira. The issue was printed on paper and has been successful to date the great impact of the event.

However, the next step would be the continuation of the same partnership, which could not be maintained in the initial terms

for the alleged exemption was intended to maintain compared to other Medical Scientific Societies requests.

However elections were held in Portuguese Anatomical Association and the new Board elected for the biennium 2014 - 2016 decided to keep an online edition, staying open to dialogue with all members, who depend on the future publication of the Archives of Anatomy.

The existence of refereed articles and abstracts of communications presented at the XLVIII Scientific Meeting of the Portuguese Anatomical Society and I Scientific Meeting of the Portuguese Anatomical Association, let us edit this issue of Archives of Anatomy, which includes a short story about the event that had place and March 22, 2014, at the Faculty of Medical Sciences, New University of Lisbon, under the chairmanship of the Director of the Department of Anatomy at the same Faculty, Professor Dr. João Goyri O'Neill. There will also be a presentation of the action plan for the biennium 2014 - 2016, by the President of the Portuguese Anatomical Association, Professor Dr. Diogo Freitas Branco Pais.

The future of Archives of Anatomy will be the desire of the Associates and we want always to be in position of Well - Serving the Cause of Anatomy and Morphological Sciences in Portugal!

Ivo Álvares Furtado
(Editor -in-Chief of Archives of Anatomy)

PLANO DE ACCÃO DIRECÇÃO AAP-SAP PARA O BIÉNIO DE 2014 – 2016

CONSOLIDAR, EXPANDIR

CONSOLIDAR

- Processo de legalização e instalação da Associação Anatómica Portuguesa: a última Direcção da SAP, Presidida pelo Professor Artur Águas, concretizou a notável constituição e o registo da AAP e respectivos Estatutos. A primeira Direcção da AAP, presidida pelo Professor João O'Neill trabalhou no sentido de assegurar as obrigações fiscais e contabilísticas da Associação, a transição dos sócios e bens da SAP para a AAP, a gestão dos associados e cobrança de quotas e a angariação de patrocínios no difícil contexto económico-financeiro do país. A presente Direcção propõe-se consolidar o trabalho realizado, contribuindo, pelas suas acções, para o desejável futuro reconhecimento, pelo Conselho de Ministros, do estatuto de Associação de Interesse Público, que nos poderá futuramente isentar do pagamento de IRC. As actividades da AAP dependem substancialmente das receitas obtidas através das quotizações e patrocínios financeiros e esta Direcção continuará a envidar todos os esforços tendentes ao aumento de associados e de outras fontes de financiamento.

- Promoção científica: A Direcção da AAP/SAP continuará a promover o patrocínio científico de cursos de pré e pós-graduação e de reuniões científicas, nacionais e internacionais, após consulta do Conselho Científico da Associação.

- Revista científica – Órgão oficial da AAP/SAP (“Archives of Anatomy”): A direcção cessante desenvolveu um importante trabalho de lançamento do projecto com a publicação, em parceria, de um número especial da revista da OM “Acta Médica Portuguesa”. Existem, neste momento artigos suficientes, incluindo os resumos dos trabalhos apresentados na XLVIII Reunião Científica da SAP e I Reunião Científica da AAP, para a publicação de um novo número que poderá ser o primeiro da revista *online*. A Direcção continuará a trabalhar no sentido de desenvolver a solução que melhor servir os interesses científicos, financeiros e outros da Associação.

- Página de Internet da AAP/SAP: A direcção precedente teve a árdua tarefa de lançar o *site* da Associação. Muito trabalho existe ainda por fazer, nomeadamente a abertura da versão em língua inglesa e a implementação da área reservada aos associados.

EXPANDIR

- Representatividade de todos os domínios com actividade morfológica: com o intuito de expandir o âmbito de actividades da AAP, bem como contribuir para a angariação de novos associados, com as consequentes repercussões pedagógicas, científicas e financeiras, a presente Direcção tem o intuito de tudo fazer para garantir a representatividade dos domínios que desenvolvam actividades científico-pedagógicas nas ciências morfológicas, nomeadamente a Medicina, a Medicina Dentária, as Ciências Farmacêuticas, a Enfermagem, as Tecnologias da Saúde, a Fisioterapia, a Engenharia Biomédica, as Belas Artes, a Medicina Veterinária, entre outras. Acreditamos que, deste modo, possamos contribuir para o crescimento e o enriquecimento da AAP.

- Terminologias Morfológicas: a Direcção anterior da AAP/SAP lançou a ideia e,

através do seu Presidente, iniciou contactos com várias escolas do país no sentido de concretizar a constituição de um grupo de trabalho para as Terminologias Morfológicas. Este grupo de trabalho vai ser chamado a duas tarefas árduas e difíceis: discutir a adopção de Terminologias Morfológicas Portuguesas e colaborar com o *Federative International Programme on Anatomical Terminologies* - FIPAT (IFAA) na revisão das Terminologias Morfológicas Internacionais. Para que se possam cumprir estes objectivos, a presente Direcção entende ser necessário constituir, no grupo de trabalho das Terminologias Morfológicas, várias secções, representativas das diferentes Ciências Morfológicas: inicialmente propõe-se a criação das secções de Anatomia (macroscópica), Histologia e Embriologia.

- Grupo de trabalho para o ensino das Ciências Morfológicas: para proporcionar a oportunidade de desenvolver actividade científica na área pedagógica das Ciências Morfológicas e para fazer face aos actuais constrangimentos, a presente direcção criará um grupo de trabalho para o ensino das Ciências Morfológicas, em articulação com o Conselho Científico da AAP.

- Relações internacionais: A Direcção cessante participou na organização do excelente *European Joint Congress of Clinical Anatomy* (Lisboa, 2013), sob a Presidência do Professor António Gonçalves Ferreira, conjuntamente com a *European Association of Clinical Anatomy* (EACA), com a *British Association of Clinical Anatomists* (BACA) e com a participação oficial da Sociedade Anatómica Espanhola (SAE) e da Sociedade Brasileira de Anatomia (SBA). Portugal detém actualmente a Presidência da EACA (António Gonçalves Ferreira) e da *European Federation for Experimental*

Morphology - EFEM (Diogo Pais). É um momento importante na história da Anatomia Portuguesa que permitirá certamente aumentar a visibilidade e a interacção da AAP/SAP com os membros das suas congéneres europeias, nomeadamente procurando implementar a organização de reuniões conjuntas da AAP/SAP com outras Sociedades Anatómicas Europeias. Simultaneamente, a Direcção procurará a contínua aproximação à Sociedade Anatómica Brasileira e, através da IFAA, a outras Sociedades e Organizações Anatómicas internacionais não europeias.

Em resumo, a presente Direcção eleita para o biénio 2014-2016 tem por objectivo maior contribuir para prestigiar a Anatomia e os anatomistas Portugueses.

Diogo Pais,
Ivo Furtado,
Maria Alexandra Brito,
Maria Alexandre Bettencourt Pires,
Jorge Celso Fonseca

(Direcção da AAP
Biénio 2014-2016)

ARTIGOS CIENTÍFICOS

Os artigos editados por extenso em *Archives of Anatomy* foram arbitrados por pares de reconhecido mérito internacional no âmbito das ciências morfológicas.

Evaluation of the efficacy of different conduits to bridge a 10 millimeter defect in the rat sciatic nerve in the presence of an axial blood supply

Avaliação da eficácia de diferentes enxertos tubulares na reparação de um defeito de 10 milímetros do nervo isquiático do rato na presença de um fornecimento vascular axial

Angélica-Almeida M¹, Casal D²; Mafra M³, Mascarenhas-Lemos L^{2,3}, Silva E⁴, Farinho A⁵, Iria I⁶, Martins-Ferreira J³, Ferraz-Oliveira M³, Videira P⁷, Vassilenko V^{8,9}; Amarante J¹⁰; Goyri-O'Neill J^{9,11}

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Resumo:

Introdução: Existem poucos estudos sobre o uso de membrana amniótica e veias autólogas como condutos para a reconstrução de defeitos nervosos periféricos. Não se sabe se a presença de um suprimento vascular axial pode melhorar o resultado obtido com estas opções. O objetivo deste trabalho foi comparar a eficácia de enxertos de membrana amniótica humana, de veia jugular externa autóloga e de auto-enxerto de nervo na reconstrução de um defeito de 1 cm de comprimento do nervo isquiático do rato, na presença de um suprimento vascular axial. **Material e Métodos:** Quarenta e cinco ratos Wistar foram submetidos à excisão de um segmento de 10 mm de nervo isquiático direito, preservando-se o plexo vascular epineural. Os ratos foram então aleatoriamente submetidos a reconstrução do defeito nervoso com um dos seguintes procedimentos: auto-enxerto do segmento excisado (Grupo A, n = 15); enxerto autólogo de veia jugular externa (Grupo B, n = 15); enxerto tubular de membrana amniótica humana (Grupo C, n = 15). Os ratos foram avaliados funcionalmente (avaliação das pegadas; eletroneurografia e força de flexão ao nível do tornozelo) e estruturalmente (avaliações histológicas e morfométricas; taxa de recuperação do peso dos músculos gastrocnémio e solhar; e marcação axonal retrógrada com True Blue) às 4, 8 e 12 semanas. **Resultados:** Os resultados médios obtidos para os parâmetros avaliados não apresentaram diferenças significativas entre os vários grupos, exceptuando uma maior recuperação de massa muscular no Grupo C relativamente ao Grupo A às 12 semanas. Os nervos reconstruídos apresentaram uma arquitetura normal, incluindo a arquitectura vascular. A membrana amniótica foi bem tolerada imunologicamente persistindo em torno do nervo até à 12^a semana. **Conclusão:** Na presença de um suprimento vascular axial local, a membrana amniótica humana e as veias autólogas são pelo menos tão eficazes como os autoenxertos nervosos na reconstrução de defeitos nervosos de 10 mm.

Palavras- Chave: Regeneração nervosa; rato; nervo isquiático; recuperação da função: transplantes; veias; membrana amniótica.

Abstract:

Introduction: There are few studies on the use of human amniotic membrane and autologous veins as conduits for reconstructing peripheral nerve defects. It is not known if the presence of a local axial blood supply may enhance the result obtained with these options. The aim of this paper was to compare the efficacy of bridging a 10 mm long nerve defect of the sciatic nerve of the rat with these options compared to the traditional nerve autograft, in the presence of a nearby axial blood supply. **Material and Methods:** Forty five female Wistar rats were submitted to excision of a 10 mm segment of the right sciatic nerve preserving the epineural vascular plexus. The rats were then randomly allocated to reconstruction of the nerve defect with one of the following: autograft from the excised segment (Group A; n=15); autologous graft from the external jugular vein (Group B; n=15); conduit built from human amniotic membrane (Group C; n=15). Rats were assessed functionally (walking track analysis; electroneurography and ankle flexion strength) and structurally (histological and morphometrical assessments; gastrocnemius and soleus muscle weight recovery; neuronal retrograde marking with True Blue) at 4, 8 and 12 weeks. **Results:** The average results obtained for the evaluated parameters did not show significant differences, apart from a greater recovery of muscle mass in the Group C relatively to Group A at 12 weeks. The reconstructed nerves showed a normal architecture and blood supply. The amniotic membrane was well tolerated as a nerve conduit, persisting around the growing nerve until the 12th week. **Conclusion:** In the presence of an axial blood supply, human amniotic membrane and autologous veins are at least as effective as autografts for bridging 10 mm nerve defects in the rat.

Keywords: Nerve regeneration; rat; sciatic nerve; recovery of function; transplants; veins; amniotic membrane.

Introduction:

A major limitation to successful repair extensive peripheral nerve injuries, is the restricted number of options available for nerve reconstruction, particularly when there is loss of nerve substance.¹⁻⁶ Moreover, the traditional nerve grafts are not only few, but are also associated with variable morbidity on the donor site.²⁻⁴ Therefore, alternatives to nerve autografts have been tried, with several artificial nerve conduits being nowadays used for bridging small nerve defects.⁷⁻¹³ However, initially these artificial nerve conduits are not vascularized, and their interior depends on neoangiogenesis for obtaining a blood supply of its own.¹⁴ This is a vital aspect of nerve repair physiology, because there is substantial evidence to support the fact that local blood supply is crucial to achieve maximal peripheral nerve regeneration, especially in cases of associated soft tissue injuries.¹⁵⁻¹⁹ Moreover, the introduction of an artificial conduit in a contaminated wound is not ideal, due to the risk of infection.¹⁻⁶ In these cases, which are frequent in clinical practice, a living nerve conduit would be preferable.

¹⁻⁶ In this sense, there have been some

experimental and clinical trials involving autologous vein grafts or amniotic membrane allografts to bridge nerve defects.²⁰⁻²⁵ Notwithstanding, these studies are scarce and show that after an initial period in which recovery is better with vein grafts or amniotic membrane allografts compared to nerve autografts, in the medium and long term, there is a similar outcome with all these options.²⁰⁻²⁶ One explanation for this finding is that initially the structural components of the vein and amniotic membrane promote vigorous nerve growth, but over time they may be associated with an insufficient blood perfusion to the growing nerve.^{11,12,20} Therefore, this paper aims to compare the efficacy of bridging a 1 cm long nerve defect of the sciatic nerve of the rat with autologous vein, with an amniotic tube conduit and with a traditional nerve autograft, in the presence of a nearby axial blood supply.

Material and Methods:

Under aseptic conditions, forty five female Wistar rats, 6 to 9 months old, weighing 200-300 g, had a 10 mm

segment of the right sciatic nerve excised in the dorsal aspect of the thigh, preserving the epineurial vascular plexus (**Fig. 1**)

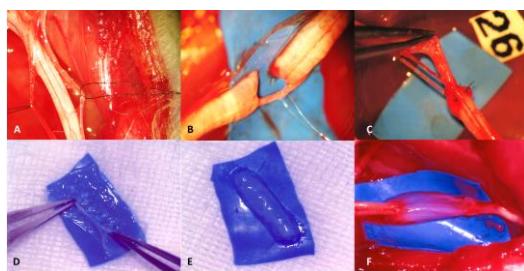


Figure 1. Photographs showing the different surgical procedures used to create the sciatic nerve defect and to reconstruct it.

A- Isolation of the extrinsic blood supply to the sciatic nerve

B- Neurotmesis of the sciatic nerve and excision of 1 cm of the nerve, preserving the extrinsic blood supply; in Group A, the excised segment of the nerve was sutured with 8/0 Nylon to its original position

C- Bridging of the sciatic nerve gap with an autologous graft of external jugular vein (Group B)

D and **E-** elaboration of a conduit with human amniotic membrane (Group C)

F- Bridging of the sciatic nerve gap with the human amniotic membrane conduit.

All lesions were made in the middle portion of the thigh. The animals were divided into 3 surgical groups: **Group A** - the nerve defect was repaired with a autograft from the excised nerve segment; **Group B** - the nerve defect was bridged with an autologous graft from the left external jugular vein of the rat; **Group C** - the nerve defect was reconstructed with a conduit of human amniotic membrane provided by the South Delegation of the Portuguese Histocompatibility Center. (**Fig. 1**) The rats were all operated on by the senior author (M.A.A) under a stereotaxic surgical microscope (Leica M651®). General anesthesia with an intraperitoneal injection of a mixture of ketamine and xylazine at doses 90 mg / kg and 10 mg / kg, respectively was used.

²⁷ Each of these groups was subdivided in 3 subgroups of 5 rats that were euthanized at 4, 8 and 12 weeks respectively.

Rats were inspected daily for signs of infection, activity levels, grooming and signs of autotomy.^{9,10} Rats' weight was evaluated weekly. Animals were assessed functionally (walking track analysis; electroneurography and ankle flexion

strength at 4, 8 and 12 weeks), structurally (morphometric assessments of the sciatic nerve proximally, distally and at the repaired zone; gastrocnemius and soleus muscle weight evaluation; assessment of restoration of correct sensory pathways with a retrograde fluorescent marker). Moreover, in each group, at 12 weeks, one rat was submitted to an injection of a Mercox ® solution through the abdominal aorta, in order to obtain vascular corrosion molds of the region of the injured nerve that were later observed with a binocular loupe and finally with a scanning electron microscope (JEOL JSM-5410®), with an acceleration voltage of 1.2-30kV, according to the protocols currently used by the authors' institution.²⁸⁻³²

Functional assessment

All animals were submitted to the evaluation of the sciatic functionality index (**SFI**) before surgery, every 4 weeks, and at the end of the experiment.^{9,10,33} The animals were placed in one end of a wooden corridor, measuring 42 cm in length and 9 cm in width, connected to a closed box in the other end of the

corridor. Millimetric paper strips were placed on the floor of the corridor.

The animals were trained to walk down the corridor. Methylene blue solution was used to paint the hindlimbs. The following measures were obtained from the footprints of both the experimental (**E**) and non-operated (**N**) hindlimbs: distance between toes 1 and 5 (toe spread – **TS**); distance between toes 2 and 4 (intermediate toe spread – **ITS**) and the print length (**PL**).^{10,33}

The Bain *et al.* formula was used to determine the SFI³³:

$$\text{SFI} = 38,3 [$$

$$(\text{EPL} - \text{NPL})/\text{NPL} + 109,5 [(\text{ETS} - \text{NTS})/\text{NTS}] + 13,3 [(\text{EIT} - \text{NIT})/\text{NIT}] - 8,8$$

A SFI of around -100 represents total hindlimb impairment, whereas a score of close to 0 indicates complete recovery of the sciatic nerve.^{10,33}

Under general anesthesia and direct visualization of the sciatic nerve, motor nerve conduction velocity (MNCV) was assessed on both limbs with the equipment Neuromatic 2000 M/C Neuromyograph ®, as described by

Varejão.¹⁰ After MNCV determination, the ankle flexion strength was evaluated on both sides by direct nerve stimulation with a neurostimulator

Plexival Medival ® set for an electric current with the following characteristics: intensity of 4,0 milliamperes; frequency of 4 Hertz; and 50 microseconds duration. The foot was on resting position and fixed with a suture line, parallel to the table, to a dynamometer transducer (Sauter FH-5®). The readings were evaluated for 30 seconds. An average value was obtained from these readings.

Structural Assessment

Immediately after functional evaluation, the sciatic nerves were fixed in formaldehyde and prepared for histological examination, using hematoxylin-eosin, Masson's trichrome stains, and immunohistochemistry for neurofilaments. Additionally, sections of the nerves were marked with CD31 immunostain, in order to highlight the endothelial lining of vessels. Concerning Group C, in one rat of each subgroup (4, 8 and 12 weeks), the nerves were stained with antibodies for human collagen-1, in order to mark the persistence of the

amniotic membrane tube. Histological preparations were observed under a digital optical microscope (Leica DMLB2®).

The density of axons, total number of fibers and the total axonal and fiber section areas were determined. To avoid “the margin effect”, the “two dimensional disector method” was applied.^{10,34} In addition, in all animals the weight of the gastrocnemius and soleus muscles of the paretic and the contralateral sides were compared by dividing these values and multiplying by 100% (percentage of gastrocnemius and soleus muscles recovery).

At 12 weeks, one animal of each group was submitted to retrograde tracing marking with True Blue (TB) to assess the restoration of correct anatomical connections of sensory pathways. At day 84, one intracutaneous injection, consisting of 12 µl of 2,5% True Blue Diaceturate (Sigma-Aldrich ®), was given in the middle portion of the sole of the right foot. Ten days later, the animals were euthanized. The dorsal root ganglia (DRG) of the spinal cord segments L1 to L4, attached to the respective spinal cord

segments, as well as coronal sections through the motor and sensory cortex³⁵ were extracted and immersed in 4% paraformaldehyde, and 10% sucrose in 0.1 M phosphate buffered saline (PBS) at pH 7.4 for 5 hours. After fixation the DRG were transferred into 15% sucrose in PBS for at least 15 hours. Finally, the DRG were transferred into 30% sucrose in PBS for at least 15 hours and then frozen in liquid nitrogen. Cryostat sections were cut at 20 µm and thaw mounted on polylysine-coated glass slides (spinal cord segments and respective spinal ganglia).^{9,36}

The DRG were then examined for fluorescence under a fluorescence microscope (Leica DMIRE 2®). Fluorescence presence was assessed on what appeared to be the largest cross section.⁹

Images were morphometrically analyzed with resort to Image J® software.^{9,10}

Statistical analysis was performed with the statistical software PASW 20.0®. ANOVA was used to compare averages between the different groups. Chi-square test was used to compare proportions. A p value under 0,05 was considered statistically significant.

All animal procedures were conducted by researchers certified in the handling of laboratory animals. The experimental protocol was approved by the Ethical committee at the authors' institution

Results:

The behavior and health of the animals was normal in all groups for the entire duration of the study. All animals had adequate activity, including grooming, and gained weight throughout the study.

None of the animals presented signs of

limb autophagy. Three animals in each group developed a chronic ulcer that did not compromise walking or other activities.

In Group A, the average SFI was -40,3; -34,1; and -24,5 at 4, 8 and 12 weeks respectively. In Group B this parameter was -38,8; -30,7; and -9,4 in the same time sets, respectively. Finally, in Group C, the average SFI was -36,6; -33,8; and -28,0 at 4, 8 and 12 weeks respectively (**Fig. 2**).

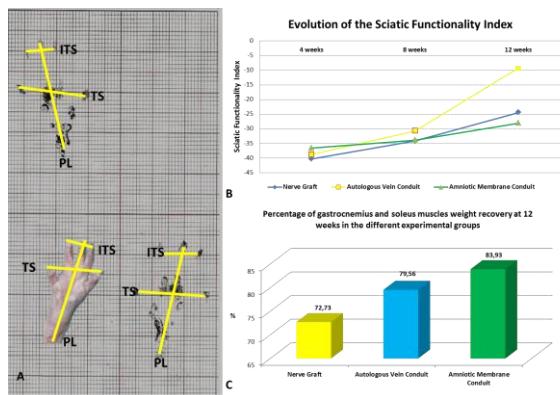


Figure 2. Functional evaluation

A- Example of a pair of footprints obtained at 12 weeks in rat submitted to nerve repair with interposition of a vein graft. For each limb the following measurements were obtained from the footprints: distance between toes 1 and 5 (toe spread – **TS**); distance between toes 2 and 4 (intermediate toe spread – **ITS**) and the print length (**PL**). The measurements were obtained in the experimental and normal limbs.

B- Comparison of the functional sciatic index in the different groups at 4, 8 and 12 weeks.

C- Percentage of gastrocnemius and soleus muscles weight recovery at 12 weeks in the different experimental groups.

These differences were not statistically significant.

The average value of MNCV in the non-operated limbs was $47,0 \pm 4,2$ m/s. At 12 weeks the average MNCV recovery rate was 60,12 % for Group A, 62,23 % for Group B and 64,01 % for Group C. The difference in the different experimental groups regarding the percentage of gastrocnemius and soleus muscles weight recovery was most marked at 12 weeks, being 72,73%, 79,56% and 83,92%,

respectively (**Fig. 2**). Only the difference between the weight of the muscles in Group C (amniotic membrane repair) and the weight of the muscles in group A was statistically significant.

The average strength in ankle flexion in the non-operated limb was $0,05 \pm 0,02$ N. The recovery rate in strength in ankle flexion was 61,32%, 58,45% and 63,17% in Groups A, B and C, respectively (**Fig. 3**).

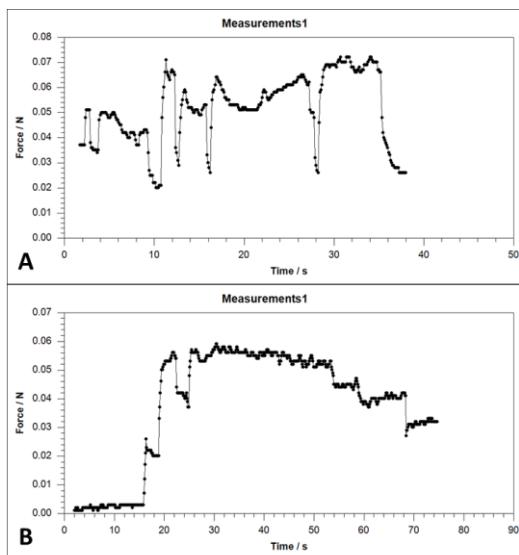


Figure 3. Example of a typical strength measure in the operated hindlimb of a rat submitted to nerve bridging with a vein graft (**A**) and the contralateral healthy hindlimb (**B**). Similar results were obtained in Groups A and C.

In all the groups at 4 weeks and beyond, all animals presented anatomical

continuity of the nerve gaps macroscopically (**Fig. 4**).

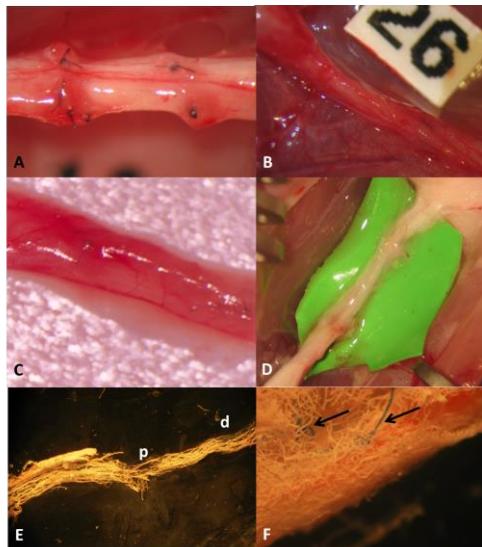


Figure 4. Photographs showing the different macroscopic aspect of the operated nerves 12 weeks after the repair with different kinds of conduits, showing anatomical continuity and a robust epineural vascular plexus

A- Nerve gap bridged by the nerve graft

B- Nerve gap bridged by the autologous vein

C- 10X magnification showing the vein conduit with a robust epineurial blood supply

D- Nerve defect bridged by human amniotic membrane

E- Vascular corrosion cast of a sciatic nerve repaired with a nerve graft preserving the epineurial blood supply; P- proximal portion of the original nerve graft; d- distal portion of the original nerve graft.

F- 20X magnification of the proximal portion of the nerve graft used to bridge the sciatic nerve defect; it is possible to observe the continuity of the epineurial plexus through the nerve repair zone; the arrows point the 8/0 Nylon stitches used in the repair.

continuity of the epineurial vascular plexus throughout the nerve repair zone (**Fig. 4**).

In addition, it was possible to observe a dense epineurial vascular plexus from that time on. Also from the fourth week onwards, vascular corrosion casts showed

Histology, showed restoration of a relatively normal nerve architecture in all the studied segments of the operated sciatic nerve (**Fig. 5**).

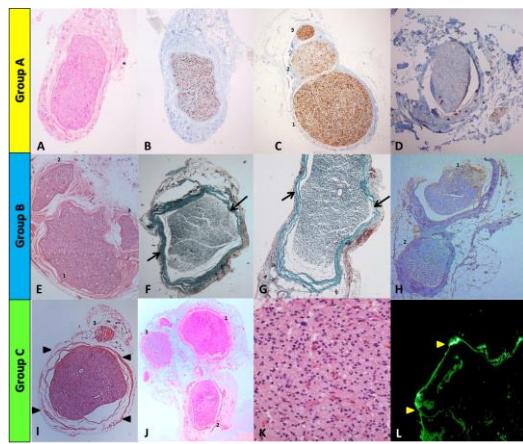


Figure 5. Microphotographs showing different histological aspect of the sciatic nerve distally to the induced nerve gap at 12 weeks

Group A- Rats submitted to sciatic nerve bridging with the nerve graft

Group B- Rats submitted to sciatic nerve bridging with the autologous external vein

Group C- Rats submitted to sciatic nerve bridging with the human amniotic membrane conduit

A- 40X magnification of a hematoxylin-eosin stained section showing restoration of a relatively normal nerve architecture

B- 40X magnification of a neurofilament stained section just distal to the nerve repair highlighting the axonal content of the nerve

C- 40X magnification of a neurofilament stained section further distally than in B (in the region of the division of the sciatic nerve in the tibial [1], common peroneal [2] and caudal sural cutaneous nerves [3]) highlighting the axonal content of the 3 nerves

D- 40 X magnification of a CD-31 stained section showing the multiple endoneurial, perineural and epineurial vessels in the distal end of the original nerve graft

E- 40X magnification of a hematoxinil-eosion stained section showing restoration of a normal nerve architecture distally to the nerve repair

F- 40X magnification of a Mason's Tichrome stained section showing the vein graft (marked by the arrows) persisting at 12 weeks

G- 100X magnification of a Mason's Tichrome stained section at the vein graft showing multiple endoneurial, perineurial and epineurial vessels

H- 40 X magnification of a CD-31 stained section at the distal ending of the sciatic nerve showing the multiple endoneurial, perineurial and epineurial vessels

I- 40X magnification of a hematoxinil-eosion stained section showing restoration of a normal nerve architecture in the distal portion of the sciatic nerve gap; the persistence of the amniotic membrane can be observed (*arrow heads*)

J- 40X magnification of a hematoxinil-eosion stained section showing restoration of a normal nerve architecture in the terminal divisions of the sciatic nerve;

K- 400X magnification of a hematoxinil-eosion stained section of the sciatic nerve in the distal end of the repaired nerve gap showing highly densely packed axons and a rich endovascular blood supply

L- 100X magnification of a immunofluorescence section marking human collagen I, documenting the persistence of the amniotic membrane (*arrow heads*) in the nerve gap at 12 weeks.

In particular, the endoneurial, perineurial and epineurial plexuses were restored in all groups. Notwithstanding, there was persistence of the vein structure in classical histological stains (hematoxilin-eosin; Masson's Trichrome). Immunohistochemistry of the nerve gaps using antibodies for human collagen 1 molecules, confirmed the presence of the amniotic membrane at 12 weeks in the Group C rats.

There were no statistically significant differences in the density of axons, total number of fibers, total axonal and fiber section areas. Retrograde neuronal marking with the True Blue fluorescent tracer stained the nerve gap, the proximal part of the sciatic nerve, and the lumbar DRG on the operated side similarly in all groups at 12 weeks (**Fig. 6**).

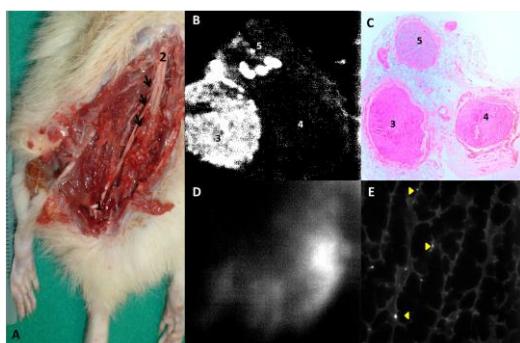


Figure 6. Typical retrograde marking with True Blue tracer.

A - Photograph of a dorsal view of the hindlimb and back showing a dissection of the sciatic nerve (**1**) through the pelvis until its origin in the sacral plexus, the lumbar dorsal root ganglia (*arrows*) and the spinal cord (**2**).

B - Photograph of a 40X magnification fluorescence image of the terminal portion of the sciatic nerve at 12 weeks after bridging the upstream nerve gap with autologous vein

3 - Tibial nerve; **4**- Common peroneal nerve; **5**- Caudal sural cutaneous nerve

C - Photograph of a 40X magnification of a hematoxilin-eosin stained section obtained at the same level of the fluorescence image showing the three terminal terminal divisions of

the sciatic nerve; it is shown to better illustrate that the fluorescence in image C is confined to the tibial and the caudal cutaneous sural nerve, that innervates the cutaneous area where the tracer had been injected.

D - Photograph of a 40X magnification fluorescence image of the dorsal root ganglion of the spinal cord segment L3 on the same side as the repaired sciatic nerve, showing retention of the tracer injected in the skin territory of that nerve.

E - Photograph of a 40X magnification fluorescence image of the granular neurons (inner granular layer IV) in the contralateral primary somatosensory area showing the labelling of the neuron cell bodies with True Blue crystals (*arrow heads*).

In all the experimental groups it was possible to identify the fluorescent marker in the cell bodies of the granular neurons (inner granular layer IV) of the contralateral primary somatosensory area (Fig.6E).

Discussion:

In rats, after neurotmesis, there is a lag period of 1-4 days, after which there is axonal outgrowth from the proximal nerve stump at a speed of up to 3 millimeters per day.⁹ These values are generally higher than those recorded in humans, where the lag phase lasts on average 9 days and the maximum speed of axonal growth is only 1 to 2 millimeters per day.⁹ The nerve defect created in this study was 10 mm long, and the length from the proximal nerve stump to the gastrocnemius muscle was around

15 millimeters.⁹ Hence, theoretically the twelve-week follow-up period used in this study is adequate for a proper evaluation of nerve regeneration in our model.⁹ In fact, according to the literature, in the majority of experiments of peripheral nerve regeneration in the rat sciatic model, assessments are made up to 12 weeks, with interim assessments at 4 and 8 weeks, as in the present work.^{9,10} This experimental work shows that both an autologous vein graft and a nerve conduit made from human amniotic membrane, associated with local blood vessels (the preserved epineurial plexus) can be used to bridge a 1 cm long nerve defect in the rat's sciatic nerve, at least as effectively as the nerve autograft, which is nowadays the standard surgical option for these defects.²⁻⁴ In fact, there were no significant differences among the

experimental groups regarding the functional and structural parameters evaluated, with the exception of the percentage of gastrocnemius and soleus muscles weight recovery, that was significantly higher in Group C than in Group A. Following the “fascicle specificity theory”, it would be better to use nerve guides or conduits rather than nerve grafts or even direct nerve sutures, when fascicle topography cannot be determined clinically, which is frequently the case.^{10,37} This is due to the fact that motor axons in the proximal nerve segment spontaneously grow preferentially towards the motor axons in the distal nerve segment, if they are not blocked by other types of axons.^{10,37,38} In this sense the venous autografts and the amniotic membrane conduits would be ideal in these circumstances.¹⁰ According to the literature, these conduits offer several additional advantages, namely: they contain important neurotropic factors; they are biodegradable; they seem to cause a very weak or absent immune response; they are flexible and therefore easily adaptable to difficult access wounds; and they are readily available or easily manufactured into different sizes and/or diameters, in order to better fit

nerve defects.^{7,11,12,20,23,39} Interestingly, the use of the amniotic membrane was first reported in the realm of peripheral nerve reconstruction in 2000.²⁰ To the best of the authors knowledge, since then only two more studies have addressed this option in the experimental setting.^{26,40} One of those studies evaluated the usefulness of photochemical tissue bonding in nerve repair. The other paper addressed the utility of including inside the amniotic membrane conduit composite nerve-muscle autografts.^{26,40}

Moreover, there have been some reports on the use of amniotic membrane to avoid and/or treat perineural adhesions.⁴¹⁻⁴⁴ These applications are supported by the present study, as immuhistochemistry of Group C animals confirmed the presence of the amniotic membrane conduit until 12 weeks postoperatively. Hence, it seems that the amniotic membrane forms a barrier around the growing axons, preventing adherence to neighboring structures.

The use of autologous vein grafts for peripheral nerve repair, on the other hand, was mentioned in the literature for the first time in 1982.⁴⁵ Since then numerous studies have addressed these conducts

both experimentally and clinically.^{21-25,39,46-52} Vein grafts have even been combined with growth factors, stem cells, pieces of muscle or nerve inside, or turned inside-out in order to better promote nerve recovery.^{21,23-25,39,46-53} However, as far as the authors could determine, this is the first time that the amniotic membrane and the autologous vein conduits have been deliberately associated with a nearby vascular axis (the preserved extraneuronal plexus) to assess the degree of nerve recovery with these conduits, compared to the traditional nerve grafts.¹¹⁻¹³ In this paper, the authors have shown that the degree of peripheral nerve recovery was as least as good with the use of human amniotic membrane and autologous vein conduits. In addition, histologically these two options were associated with a morphological structure distally to nerve repair and even in the bridged nerve defect that was almost identical to a normal nerve, particularly in what pertains to peripheral nerve blood supply.⁵⁴ This lends further support to the clinical use of these two conduits in bridging small peripheral nerve defects. However, it remains to demonstrate if longer and wider nerve defects could be

successfully addressed in a similar fashion. This question warrants further studies.

Conclusion:

This experimental work has shown that both an autologous vein graft and a conduit made of human amniotic membrane could be used to bridge a 1 cm nerve defect in the rat's sciatic nerve, at least as effectively as the conventional nerve graft used, in the presence of nearby axial blood supply.

The resulting reconstructed nerve using these two autologous conduits showed a typical architecture, including a normal distribution of endoneurial, perineurial and epineurial vessels.

The amniotic membrane was well tolerated as a nerve conduit persisting around the growing nerve until the 12th week. This may help avoid nerve adherences.

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Angiomorphological comparison of the sciatic nerve of the rat and the human median nerve: implications in experimental procedures

Comparação angiomorfológica do nervo isquiático do rato e o nervo mediano humano: implicações nos procedimentos experimentais

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Resumo:

Introdução: As lesões do nervo mediano são frequentes clinicamente. O nervo isquiático do rato é o modelo mais utilizado para o estudo destas lesões. O objetivo deste trabalho foi investigar se a vascularização destes dois nervos é semelhante, uma vez que o suprimento vascular é considerado um factor fundamental para a reparação nervosa quer no contexto experimental, quer clínico. **Material e Métodos:** Trinta ratos adultos foram estudados quanto à vascularização do nervo isquiático. Estes dados foram comparados com os de uma análise semelhante efectuada nos nervos medianos de sete cadáveres humanos. As técnicas utilizadas foram a injecção intravascular e dissecção, bem como técnicas de microscopia óptica e microscopia electrónica de varrimento.

Resultados: Tanto o nervo mediano humano como o nervo isquiático do rato recebiam vários ramos dos vasos próximos e dos vasos sanguíneos que forneciam os músculos vizinhos, especialmente onde os nervos davam ramos para os músculos. A principal diferença entre estes dois nervos foi o padrão de distribuição de vasos para o plexo epineural. A sua microvascularização era muito semelhante. **Discussão:** A principal diferença entre este estudo e outros semelhantes foi a constatação da importância dos vasos que suprem os músculos vizinhos para a vascularização dos nervos isquiático do rato e mediano humano. **Conclusão:** A vascularização dos nervos isquiático do rato e mediano humano é semelhante, o que suporta a utilização do rato como modelo experimental de lesões do nervo mediano humano.

Palavras-chave: Nervos periféricos; Lesões dos nervos periféricos; Anatomia e histologia; Ratos; Cirurgia; Vasos sanguíneos; Retalhos cirúrgicos.

Abstract:

Introduction: Median nerve lesions are frequent clinically. The sciatic nerve of the rat is the most commonly used model for the study of these lesions. The aim of this work was to investigate if the blood supply to these two nerves was similar, as this is considered fundamental for nerve repair both experimentally and clinically.

Material and Methods: Thirty adult rats were studied regarding the blood supply to the sciatic nerve. These data were compared with a similar analysis in the median nerves of 7 human cadavers. Intravascular injection and dissection under an operating microscope, as well as optical microscopy and scanning electron microscopy techniques were used. **Results:** Both the human median nerve and the rat sciatic nerve received multiple vascular branches from nearby vessels, and from the blood vessels that supplied the neighboring muscles, particularly in places where the nerves gave off branches to those muscles. The main difference between these two nerves was the pattern of feeding vessels to the epineurial vascular plexus. Their microvascular blood supply was very similar. **Discussion:** The main difference between our work and some related reports was the finding in the specimens we analyzed of the importance of the vessels that supply the neighbor muscles to the blood supply of both the rat sciatic nerve and the human median nerve. **Conclusion:** The human median nerve and the sciatic nerve are very similar in terms of blood supply. This supports the use of the sciatic nerve of the rat as a good experimental model for lesions of the human median nerve.

Keywords: Peripheral Nerves; Peripheral Nerve Injuries; Anatomy and histology; Rats; Surgery; Blood Vessels; Surgical Flaps.

Introduction:

Upper limb nerve lesions are frequent, being most commonly caused by nerve compression by surrounding structures or by nerve transection.¹⁻³

Among nerve compressions the most common are those affecting the median nerve, particularly at the carpal tunnel, causing carpal tunnel syndrome. This pathology is estimated to have a lifetime incidence of at least 3% of the general population.^{1,4,5} In the United States of America, carpal tunnel syndrome is associated with around 90% of outpatient visits due to nerve-related dysfunction, corresponding to almost 13% of total outpatient visits to family doctors' offices.^{1,4} In addition, not only are median nerve compression syndromes highly prevalent, but they are associated with significant personal distress and with important socioeconomic consequences.^{1,4,5} For example, after surgical treatment for carpal tunnel syndrome, more than 10% of patients permanently stop working and an even higher proportion report impaired function in work and/or leisure activities.³ Moreover, nerve sections,

particularly of the median nerve, although much less frequent than nerve compressions, still represent 3% of upper extremity lesions referred to Hand Units.³ These lesions have a worse prognosis than nerve compressions and are associated with a significant burden to afflicted individuals and to the society.^{2,3,5} In Sweden, for example, it was determined that an employed person with a median nerve section in the forearm represents a 51.238 euros cost to society in the first few years after the lesion.^{6,7}

Therefore, it comes as no surprise that numerous studies have been conducted to permit a better understanding and treatment of the disorders of the peripheral nerves, and in particular of the median nerve.^{2-4,8,9} The rat has been the most commonly used experimental animal model.⁹⁻¹² In this species, the sciatic nerve has been traditionally used as a surrogate of the typical human nerve, particularly of the human median nerve.^{9,10,13} It has been largely assumed that these two nerves (the human median nerve and the rat sciatic nerve) are much alike in most important aspects, namely in

terms of nerve repair physiology.^{2,13-15} However, knowledge of the degree of resemblance of the vascular supply to these two nerves is lacking.^{9,15} This in turn has potential significant implications while studying new strategies for median nerve repair using the model of the rat sciatic nerve.¹⁶⁻²⁰ Moreover, a sound knowledge of nerve blood supply is of paramount importance both experimentally and clinically, namely in planning and executing free or pedicled nerve flaps or composite flaps including nerves.^{16,20,21}

Therefore, the aim of this work was to systematically compare the general macro and microvascular supply of the rat sciatic nerve with that of the human median nerve, giving particular importance to aspects that may be of experimental interest.

Material and Methods:

Thirty adult rats weighing between 300 and 350 g were subjected to general anesthesia by intraperitoneal injection of a mixture of ketamine and xylazine at doses 90 mg / kg and 10 mg / kg,

respectively.²² Animals were submitted to a median laparotomy and then euthanized by placing a catheter inside the abdominal aorta and another inside the caudal vena cava, and by exsanguinating animals and replacing the blood with heparinized saline (50 units/ml).

In 25 animals a colloidal suspension of barium sulphate (Micropaque ® - Nicholas - Lab) mixed with 10% commercial gelatin (in equal parts), and stained with a red or blue pigment (Pigment Tintolac Super ®, Robialac) was injected intravascularly after performing a median laparotomy. The red-colored solution was injected into the abdominal aorta and the blue-stained solution in the caudal vena cava, according to the techniques currently in use at our institution.²³⁻²⁷

Rats were dissected with the use of a binocular surgical microscope (Leica® M 651). The constitution and distribution of the sciatic nerve and its branches, as well as the origin and termination of the arteries and veins that supplied those nerves was registered. Subsequently, in 20 of the 25 injected

rats, nerves were removed and made diaphanous, according to the technique developed by Spalteholz and subsequently modified by Esperança-Pina and Goyri O'Neill.²⁸⁻³¹ These nerves were observed under a binocular loupe, studying their vascularization. In addition, in 5 of the intravascularly injected rats, the nerves were fixed in formaldehyde and prepared for histological examination, using hematoxylin-eosin and Masson's trichrome stains. Additionally, sections of the nerves were marked with CD31 immunostain, in order to highlight the endothelial lining of vessels.

Finally, in 5 rats a Mercox ® solution was injected through the abdominal aorta, in order to obtain vascular molds that were later observed through scanning electron microscopy, according to the protocols currently used at our institution.²³⁻²⁷

In 7 human cadavers, a colloidal suspension of barium sulphate (Micropaque ® - Nicholas - Lab) mixed with 10% commercial gelatin (in equal parts), and stained with a red pigment (Pigment Tintolac Super ®, Robialac) was injected at the subclavian artery supraclavicularly.²³⁻²⁷ The

median nerves were dissected under loupe magnification from their origin in the axilla until their terminal branches in the hand. The vessels supplying the median nerve were registered.

Subsequently, in 5 of these 7 cadavers, median nerves were removed and made diaphanous, according to the technique mentioned above.²⁸⁻³¹

These nerves were dissected under surgical loupes, studying their vascularization. Moreover, in 2 of the cadavers previously injected intravascularly with a colored solution, the median nerves were fixed in formaldehyde and prepared for histological examination, using hematoxylin-eosin and Masson's trichrome stains. Additionally, sections of the nerves were marked with CD31 immunostain, in order to highlight the endothelial lining of vessels. Lastly, in 2 cadavers a Mercox ® solution was injected through the subclavian artery, in order to obtain vascular molds that were later observed through scanning electron microscopy, according to the protocols currently used at our institution.²³⁻²⁷

All animal procedures were conducted by researchers certified in the handling of laboratory

animals. The experimental protocol was approved by the Ethical committee at the authors' institution.

Results:

Both the human median nerve and the rat sciatic nerve received multiple vascular branches from nearby vessels, particularly from those with a parallel path to the nerve, even if transitorily (Figs. 1 to 4).

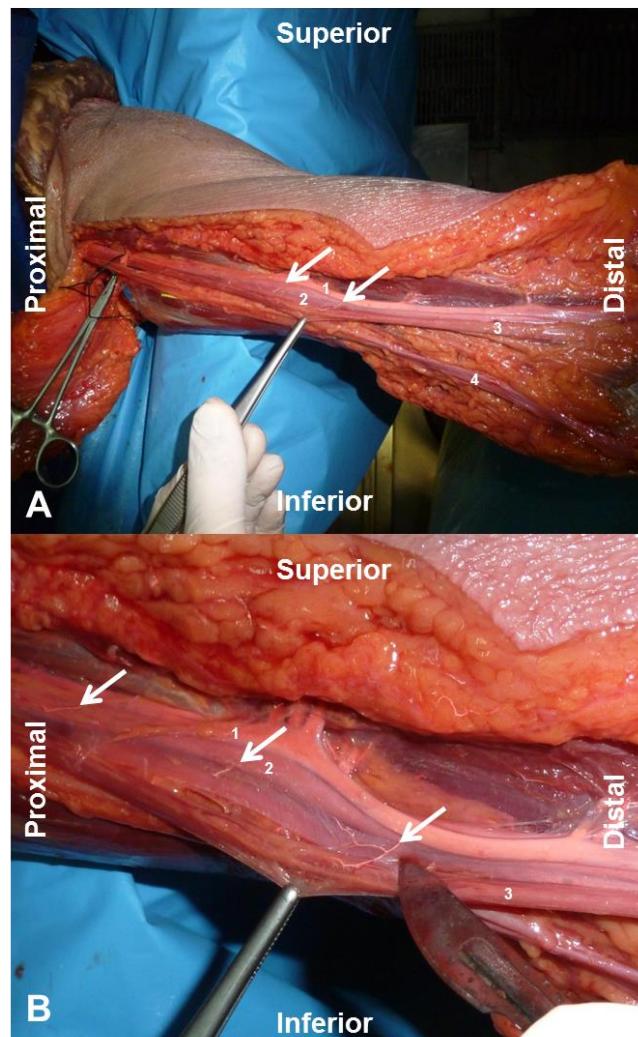


Figure 1. Photographs of the medial aspect of a dissection of a right arm showing multiple branches from the brachial artery to the median nerve (**A**). **B** shows a closer view of some of those branches.

1- brachial artery; 2- brachial vein; 3- median nerve; 4- basilic vein; arrows- branches from the brachial artery to the median nerve.

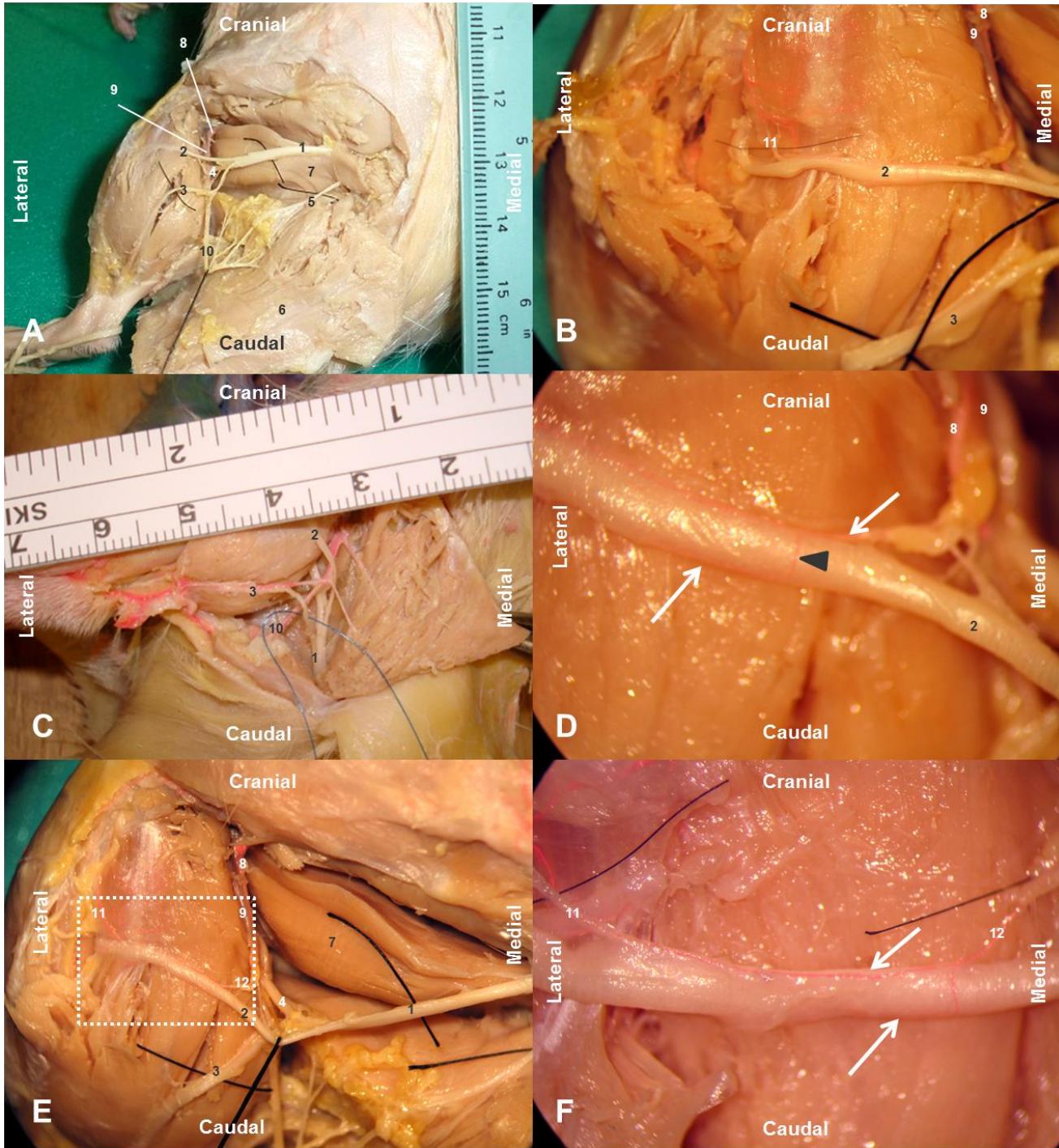


Figure 2. Photographs of dissections of the dorsal aspect of the hindlimb of rat specimens, showing the normal distribution of the sciatic nerve and its blood supply.

A. Path and distribution of the sciatic nerve

- B.** Photograph of one of the terminal branches of the sciatic nerve illustrating the contribution to the epineurial plexus of branches from large neighboring vessels, in this case, the popliteal vessels, and branches from vessels supplying nearby muscles
- C.** Photograph highlighting the topographical proximity between the sciatic nerve and major vessels in the dorsum of the hindlimb that provide branches to this nerve
- D.** A 10x magnification photograph showing the epineurial vessels with a transverse anastomotic branches between them (arrow head)
- E** and **F.** Example of equal contributions to epineurial vessels of branches from large neighbor vessels and vessels given off by blood vessels supplying muscles innervated by those nerves. These latter vessels are seen traveling retrogradely with the nerve. The photograph in **F** corresponds to the area inside the dotted box in **E** with a 25X magnification.
- 1- sciatic nerve; 2- common peroneal nerve; 3- caudal sural cutaneous nerve; 4- tibial nerve; 5- muscular branch of the sciatic nerve; 6- gluteus muscles; 7- adductor muscles; 8- popliteal artery; 9- popliteal vein; 10- caudal femoral artery and vein; 11- vessels to the nerve from nearby muscles

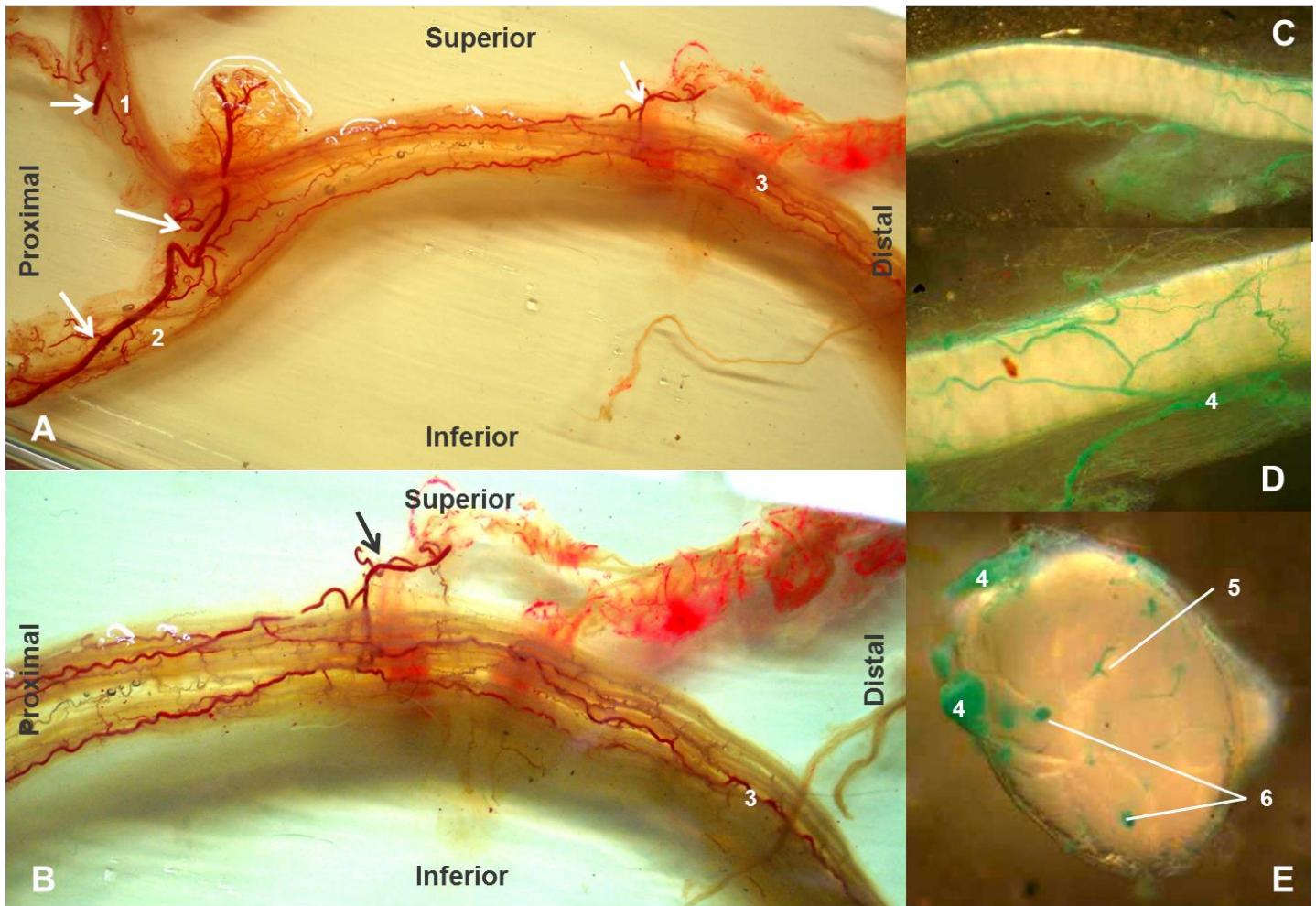


Figure 3. Photographs of the human median nerve (**A** and **B**) and the rat sciatic nerve (**C**, **D** and **E**) made diaphanous and previously injected with an intravascular colored contrast.

A and **B**. Multiple feeding vessels (arrows) are seen supplying the epineurial vessels of the median nerve.

C. A 4X magnification of the sciatic nerve of the rat shows the longitudinal epineurial vascular plexus.

D. A 10X magnification shows multiple transverse and oblique anastomotic vessels on the surface of the epineurium.

E. A 10X magnification of a transverse section of the sciatic nerve of the rat showing the topographical relation between the epineurial, the perineurial and the endoneurial vessels.

1- Lateral root of the median nerve; 2- media root of the median nerve; 3- median nerve; 4- epineurial vessels; 5- perineurial vessels; 6- endoneurial vessels.

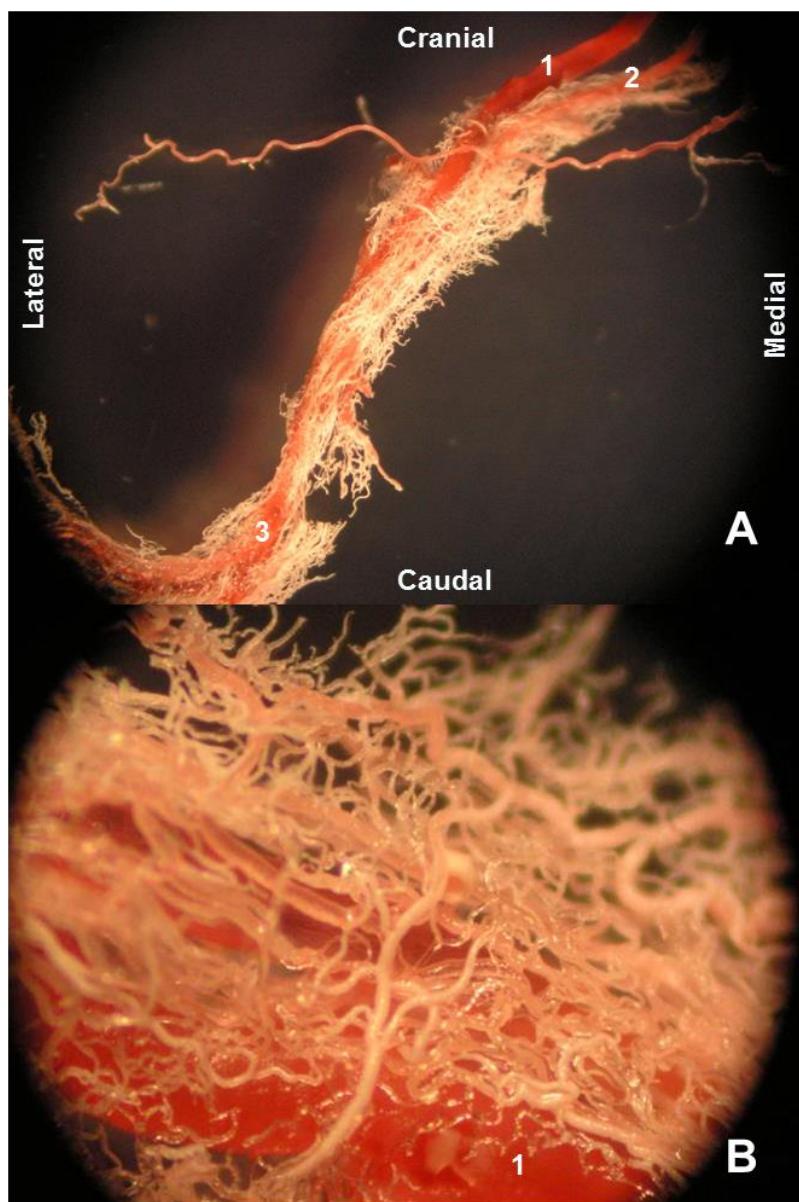


Figure 4. Photographs of a corrosion vascular cast of the sciatic nerve of the rat showing the main blood supply to the nerve.

A. A 10x magnification photograph shows the large sciatic comitans artery (1) seen following the path of the nerve after originating from the caudal gluteal artery. An anastomotic artery (2) that originates in the medial aspect of the thigh from the medial circumflex femoral artery can also be observed supplying the nerve. Finally, the popliteal artery contribution (3) to the blood supply to the sciatic nerve is also clearly visible near its terminal division.

B. A 40X magnification photograph of the middle portion of the sciatic nerve shows the perineural and endoneurial vessels originating from the sciatic comitans artery. These vessels can also be seen profusely anastomosed among themselves forming a longitudinal plexus along the nerve's major axis.

These vessels supplied the epineurial vascular plexus with several branches along the length of the nerve in a relatively variable manner. Additionally, both the human median nerve and the rat sciatic nerve received branches from the blood vessels that supplied the neighboring muscles, particularly in places where the nerves gave off branches to those muscles. In the latter circumstances, it was very frequent to observe blood vessels escorting the nerve branches to the muscles, with an opposite direction to the nerves, and ending up by establishing anastomosis with the epineurial vascular plexus (**Fig. 2**).

The median nerve originated in front of the axillary artery by the confluence of the medial and lateral roots. From there on until its ending in its terminal branches in the hand, it received multiple branches from the axillary, brachial, radial, ulnar, inferior ulnar collateral, recurrent ulnar, and from the anterior interosseous vessels and from the palmar arches in the hand (**Figs. 1 and 3**). Since its origin from the medial and lateral roots, the multiple vessels that supplied the median nerve formed continuous intraneuronal plexuses that followed the entire length of the nerve (**Fig. 3**). According to Taylor's classification of nerve blood supply,³² the median nerve followed a type A

pattern in the arm, a type E pattern in the proximal and distal forearm, and a type C in the middle forearm. The anterior interosseous nerve and the digital palmar collateral nerves originating from the median nerve were followed in parallel by homonymous branches and thus presented a type A pattern.

The sciatic nerve of the rat originated from the sacral plexus where it received branches from the sacral and from the cranial and caudal gluteal vessels. The nerve reached the dorsal aspect of the thigh through the sciatic notch. In this compartment the nerve received large accompanying vessels from the caudal gluteal vessels that give off the vessels comitans of the sciatic nerve (**Figs. 2 through 4**). In the middle aspect of the thigh the sciatic nerve

received a large vascular bundle from the medial circumflex femoral vessels, with a slightly smaller caliber than the sciatic nerve vessels comitans proper (**Fig. 4**). The popliteal vessels originated vessels that supplied the distal portion of the sciatic nerve and its terminal branches around the knee joint (**Figs. 2 to 4**). Hence, following Taylor's classification of blood supply, the sciatic nerve of the rat in the dorsal aspect of the thigh would be classified as a type D nerve.^{32,33}

...Histologically, the most striking difference between the human median nerve and the sciatic nerve of the rat was that the former was polyfascicular, whereas the latter was oligofascicular (**Fig. 5**).

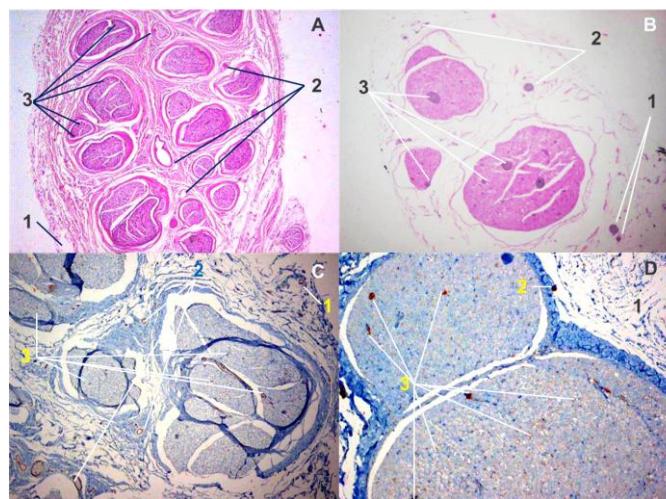


Figure 5. Optical microscopy photographs of the blood supply to the human median nerve (**A** and **C**) and to the sciatic nerve of the rat (**B** and **D**).

A and **B**. Photograph of hematoxylin-eosin stained sections of the human median nerve (**A**) and of the rat sciatic nerve (**B**) showing the epineural (1), perineural (2) and endoneurial blood vessels (3) [4X magnification].

C and **D**. Photographs of CD-31 immunostained sections of the human median nerve (**C**) and of the rat sciatic nerve (**D**) showing the epineural (1), perineural (2) and endoneurial blood vessels (3) [10X magnification].

However, the microvascular supply to these two nerves was very similar (**Figs. 5 and 6**). Both these nerves presented relatively few, large epineurial blood vessels that travelled along the main axis of the nerve. These vessels presented numerous anastomoses among themselves throughout the course of the nerve. The epineurial vessels gave off

large oblique branches that supplied the perineural and endoneurial plexuses. These two plexuses were very dense, particularly the later, forming a robust capillary net throughout the entire course of both nerves (**Fig. 6**). The perineural vessels showed a predominantly longitudinal path (**Fig. 6**)

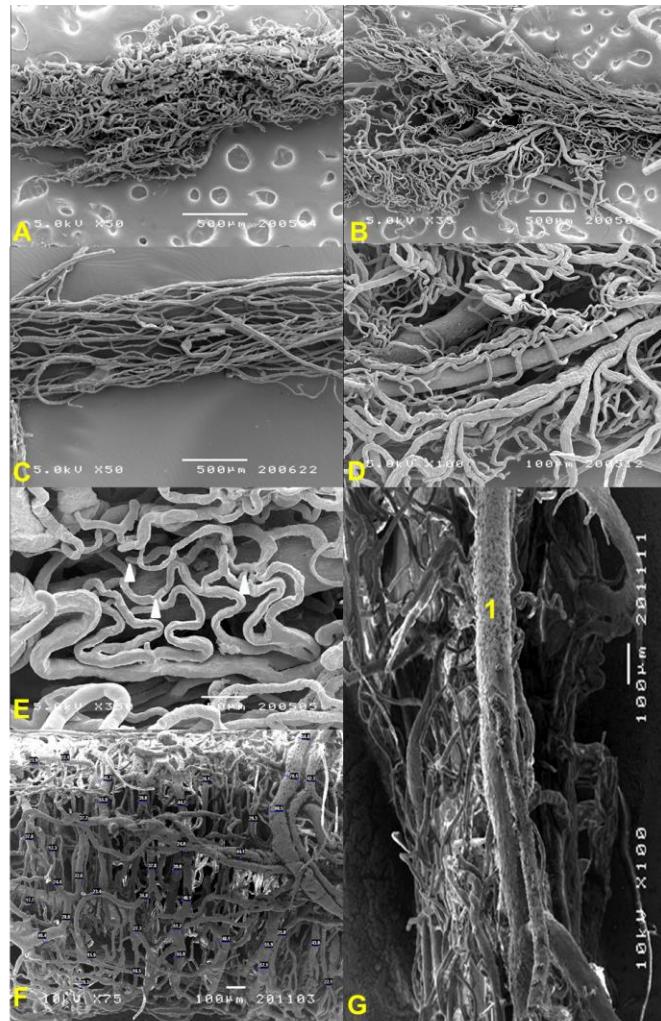


Figure 6. Scanning electron microscopy images of the blood supply to the sciatic nerve of the rat (**A** through **E**) and to the human median nerve (**F** and **G**).

A, B and C. Proximal (**A**) distal (**B**) and intermediate portion (**C**) of the sciatic nerve of the rat showing the predominantly longitudinal arrangement of the blood vessels of the epineurium and perineurium along the major axis of the nerve and its terminal branches.

D. A closer look at the sciatic nerve of the rat shows the endoneurium vessels forming a dense vascular plexus that receives multiple branches from larger vessels coming obliquely from the perineurial vessels.

E. A higher magnification of the sciatic nerve of the rat shows multiple anastomoses (arrow heads) between the endoneurial vessels.

F and **G.** A portion of the human median nerve at the arm level showing large epineurial vessels (1) giving off oblique branches that supply the perineurial and the endoneurial vessels in a manner analogous to that seen in the rat sciatic nerve.

Discussion:

Comparing our series of 30 dissected rats and 7 human cadavers, with the 34 dissected rats reported by Bell and Weddell (1984), and with the four cadavers used in Taylor's study (2006) of the blood supply to the nerves of the upper limb, we conclude that the present series is one of the largest in the literature, particularly comparing the blood supply of the sciatic nerve of the rat and that of the human median nerve.^{21,32,34,35} Interestingly, most of our findings regarding the composition of the blood vessels of these nerves are coincidental with these and other reports.^{9,16,21,33-40}

One of the main limitations of our work was that we were not able to precisely assess the location

and measure all the individual blood vessels supplying the rat sciatic nerve and the human median nerve. This was due to the extreme fragility and small diameter of many of these vessels, which precluded preservation of their integrity during dissection. This difficulty has been reported by many authors studying nerve blood supply.^{16,21,41,42} However, our goal to study the general macro and microvascular supply of the rat sciatic nerve and that of the human median nerve was, nevertheless, achieved. This knowledge is considered the most relevant for experimental purposes.^{16,17,19,21,43}

The main difference between our study and many of the classical studies performed in the rat sciatic nerve was the finding in the specimens we analyzed

of the importance of the vessels that supply the neighbor muscles to the blood supply of both the rat sciatic nerve and the human median nerve. This fact has been clearly shown recently by Taylor's group in human cadaveric specimens, but had been largely been ignored by other authors, particularly in the context of rat anatomy studies.^{2,9,16,21,32-35,44}

These findings can be of great clinical and experimental value as many clinical problems that affect the median nerve, such as compressive syndromes are strongly associated with nerve ischemia.^{3-5,45,46} For example, these anatomical data suggest that while performing neurolysis, surgeons should avoid injuring the small vessels that accompany nerve branches into adjacent structures.⁴⁷ Another consequence of this information is that, contrarily to traditional teaching, while attempting to advance the proximal and distal nerve stumps to bridge a nerve gap, it is probably unwise to exclude the small muscular branches given off by the two nerve ends in the vicinity of the defect, as this also excludes blood vessels nourishing the nerve segments.^{8,47} This

may in fact condition a worsening of ischemia, condemning nerve repair to a lower success rate than that could be obtained by using nerve flaps (that is to say nerve grafts with a blood supply of their own).⁴⁸ This could, in turn, help explain the better results reported by several authors when using nervous flaps to bridge nerve defects in situations of relative ischemia, as in the case of large areas of fibrosis or areas that have been previously submitted to radiotherapy.^{16,21,49}

Taylor's classification was initially devised for assessing the possibility of transferring nerve as free flaps.³² However, it is becoming increasingly used as a practical means of conveying the general blood supply of nerves. In this classification, type A refers to an unbranched nerve supplied segmentally by a vessel in parallel; type B to a branched nerve supplied in the same manner as in type A; type C to an unbranched nerve with a long vascular pedicle coursing in the epineurium; type D to an unbranched nerve with multiple vascular pedicles of different source vessels; and type E to a branched nerve with a vascular supply analogous to type D.

According to this classification, in the present study, the median nerve followed a type A pattern in the arm, a type E pattern in the proximal and distal forearm, and a type C in the middle forearm. The sciatic nerve of the rat in the dorsum of the thigh, where this nerve is most commonly used experimentally, was a type D nerve. The corollary of this is that the blood supply to the epineurial plexus of the sciatic nerve of the rat, in the dorsal aspect of the thigh, where it is most commonly used experimentally, is similar to that of the human median nerve in the forearm, but significantly different from that of the human median nerve in the arm and hand. This should be taken into account when performing experimental procedures that entail dissection of the sciatic nerve of the rat for nerve advancement or local or distant nerve transfers. Particularly, when extrapolation of the results to the human species is the ultimate goal.

Overall, the present work concurs with the previously published literature that the rat sciatic nerve is similar to the human median nerve in most of the basic anatomical and physiological aspects, including the general pattern of blood supply.⁹ The

main difference refers to the pattern followed by the feeding vessels to the epineural plexuses of these two nerves. These similitudes and differences should be taken in consideration while planning, executing and interpreting experimental procedures in the rat.

Conclusion: The human median nerve and the rat sciatic nerve are very similar in terms of blood supply. The main difference pertains to the pattern followed by the epineurial feeding vessels of these two nerves. This homology supports the use of the sciatic nerve of the rat as an adequate experimental model for lesions of the human median nerve.

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Preservation of organs in glycerol by vacuum processing

Preservação de orgãos em glicerol através do processamento a vácuo

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RESUMO

Objectivos: Perante a dificuldade em obter cadáveres para ensino e investigação o objectivo deste trabalho foi o de testar uma técnica alternativa pouco dispendiosa para preservar órgãos dissecados previamente, para melhorar o ensino prático no futuro. **Material e Métodos:** Foram usadas 40 peças de cadáveres, oito pernas e dois pés, e 30 órgãos: corações, fígados, rins e cortes de cérebros. Após dissecção as peças foram fixadas em aldeído fórmico a 10%. Seguidamente foram branqueadas ligeiramente em peróxido de hidrogénio. A desidratação iniciou-se com a lavagem em álcool a 95° e prosseguiu com impregnação em acetona no vácuo. Foram depois impregnadas em xilol. Finalmente efectuou-se a embebição em glicerol, no vácuo. **Resultados:**

As peças anatómicas preservadas em glicerol no vácuo mantêm a relação dos elementos anatómicos que as constituem entre si e não apresentam alterações significativas na coloração natural dos tecidos. As peças ficam flexíveis e podem ser manipuladas. **Conclusão:** Esta técnica de impregnação em glicerol é um processo eficaz, pouco demorado e pouco dispendioso para conservar órgãos intactos ou dissecados constituindo uma ferramenta válida para o ensino prático da Anatomia, nomeadamente em escolas onde há poucos cadáveres disponíveis para tal.

ABSTRACT

Objectives: Given the difficulty to obtain cadavers for teaching and research purposes, the objective of this work was to experiment an alternative inexpensive technique to preserve previously dissected organs, enabling a better practical teaching in the future. **Material and Methods:** Forty corpses pieces were used, namely eight legs, two feet and 30 organs: hearts, livers, kidneys and brains. After dissection, the pieces were fixed in 10% aldehyde formic. Then they were slightly whitened in 20 volumes of hydrogen peroxide. Dehydration began with washing in 95° alcohol, followed by acetone soaking under vacuum. Afterwards they were impregnated with xylol. Finally vacuum glicerol soaking was performed. **Results:** Organ preservation in glycerol by vacuum processing maintains the normal relation between the anatomical structures. Additionally, the natural color of the tissues is almost maintained. The pieces proved to be flexible, enabling its manipulation. **Conclusion:** Glycerol impregnation revealed to be an efficient, not time consuming and inexpensive technique useful for intact and dissected organ preservation. It is a valid technique for Anatomy teaching, namely in medical schools with lack of cadavers.

Keywords: Glycerol, organ preservation

INTRODUCTION

Anatomy is a fundamental basic science for undergraduates and postgraduate students, especially in surgery. The dissected cadaver remains the most powerful means of presenting and learning anatomy as a dynamic basis for solving problems presented by the patient¹. The manual skills learnt in the dissecting room are essential in almost every branch of the medical profession. Hands-on teaching on real cadavers is the first experience with the structural organization of the human body enabling a real understanding of the three-dimensional configuration of patient's anatomy². Anatomical dissection is also important to demonstrate the high frequency of normal anatomical variations. The lack of knowledge of these anatomical variations is believed to be responsible for nearly 10% of bad clinical practice³. In spite of some hundreds of donors, in the Faculty of Medicine of the University of Coimbra there are few cadavers available for teaching and research (only six new bodies each year). This difficulty has been compensated by the preservation of natural parts previously dissected, using classic techniques

already very familiar: paraffin soaking⁴, dehydration⁵, corrosion of vascular casts⁶⁻⁷⁻⁸, plastination⁹⁻¹⁰⁻¹¹⁻¹²⁻¹³. The pedagogical value of these natural models is invaluable and higher than that of the plastic models or Computer Assisted Instruction. However while some of these techniques are very time consuming and expensive, others provoke great deformation in the organs, or require them to be kept in preserving products, which limits its observation and study. The objective of this work is to create an alternative technique to preserve organs previously dissected avoiding the previously mentioned complications, in order to have a better practical teaching in the future.

MATERIAL AND METHODS

In this study we included 40 pieces of corpses previously dissected, namely eight legs and two feet obtained after amputation due to chronic ischemia at University Hospital of Coimbra, after informed consent of their patients. The 30 remaining pieces included hearts, livers, kidneys (previously injected) and slices of brains and were

obtained during autopsies on Coimbra's Delegation of the National Institute of Legal Medicine, after consultation with the "RENNDA" (national register of non-Donors).

After being dissected on fresh condition, the pieces have undergone fixation by immersion in 10% formic aldehyde, 10 times greater than the tissue volume, during 40 hours on average, with a minimum of 24 and a maximum of 72 hours, depending on the thickness of the organs. Then they were slightly whitened by immersion in a solution containing 20 volumes of hydrogen peroxide during 24 hours on average (minimum 18 and maximum 36 hours) with the aim of mitigating the gray color produced by formic aldehyde.

Dehydration process began with washing in 95% alcohol and it continued with immersion in 95% alcohol during 12-24 hours. After this procedure the organs were submitted to freeze substitution by immersion in acetone at -25° C during 24-48 hours. Dehydration procedure ended with vacuum assisted acetone soaking. In order to

achieve complete dehydration several passages (2 to 4) with pure acetone were undertaken. Each passage ended in vacuum when liquid kept bubbling. The complete impregnation was attested by densitometry. Afterwards, the organs were soaked in xylene, also in vacuum and attested by densitometry. Finally we made the soaking in glycerol, once again in vacuum and attested by densitometry also. The organs were dried in room atmosphere.

This organ preservation technique was implemented four years ago.

RESULTS

In all organs preserved with this technique relative dimensions of its various constituents were maintained. The pieces were easily manageable allowing its use by students and anatomists in classes and research projects without modifying its physical properties. In spite of slight whitening when treated with hydrogen peroxide, all pieces presented an acceptable final color (Fig. 1 a 6).



Figure 1. Larynx and trachea: A- anterior view; B- posterior view



Figure 2. Liver



Figure 3. Kidney showing injection of renal veins



Figure 4. Brain section



Figure 5. Foot - dorsal view



Figure 6. Leg and foot - anterior and lateral view

In addition their consistency was relatively smooth and they were quite malleable and resistant to manipulation. Four years after being subjected to impregnation in glycerol, the organs remain

unchanged, sturdy and in very good conditions to be handled and studied without damage.

Although these advantages, all pieces presented a reduction of volume due to tissue

dehydration, exception made to the bone tissue which obviously remained unchanged.

DISCUSSION

One of the usual concerns of Anatomists is the preservation of bodies or parts of bodies for teaching and research purposes, since they perpetuate beautiful, informative and didactic dissections and compensate the difficulty in obtaining bodies. In the past decades multiple techniques for organ and corpse preservation have been used, namely: paraffin soaking, dehydration, corrosion of vascular casts and plastination¹¹⁻¹³⁻¹⁴⁻¹⁵⁻¹⁷. However there is still no ideal technique.

All processes begin with fixation of the pieces which is responsible for inevitable changes, namely in shape, volume, weight and color⁶⁻¹⁸⁻¹⁹⁻²⁰⁻²¹. In fact all techniques deform the organs.

Some of these methods, such as plastination, are particularly expensive since they require special equipment as well as specific chemical products¹¹⁻¹³.

To make the dehydration Gunther Von Hagens¹³ uses one of two methods: stepwise dehydration in graded ethanol and freeze substitution with acetone. Stepwise ethanol dehydration causes considerable shrinkage (roughly 50%, according to Schwab and V. Hagens)²² and is time consuming. The standard dehydration procedure for plastination is freeze substitution in acetone at -25° C according to Gunther Von Hagens¹³. This method saves time, requires less labor compared with ethanol dehydration, and causes only minor tissue shrinkage.

The technique of waxing¹⁹ is also easy to perform and inexpensive but has one disadvantage: the need to impregnate the piece in an oven at 50°, corresponding to the melting point of paraffin. This temperature is responsible for the unsightly dark brown color parts that usually arise in muscle tissue. Impregnation with glycerol eliminates this problem. In addition waxing turns the preserved parts rigid while glycerol makes them soft and pliable, which is an advantage in specific organs such as the larynx and trachea.

The technique that we present in this article is easy to perform and is not expensive. It just needs a vacuum chamber, whose dimensions depend on the size of the pieces to be processed, coupled to a vacuum pump. Regarding the chemical products this technique uses formic aldehyde, hydrogen peroxide, alcohol, acetone, xylene and glycerol, all of them simple to acquire. The final step of this procedure consists in impregnation in glycerol, an organic compound belonging to the polyol (sugar alcohol) group. It is an odorless, colorless, viscous chemical that is liquid at room temperature. Glycerol has three hydrophilic groups that are responsible for its solubility in water and its hygroscopic nature²²⁻²⁴. Glycerin refers to the commercial form of the product with purity above 95%. Application of vacuum accelerates dehydration and impregnation by glycerol allowing to obtain pieces in an average of eight days. Without vacuum dehydration of the liver takes around 3 months¹⁸⁻¹⁹.

With the proposed technique, after a four year period all preserved parts are in perfect condition, seemingly unchanged and continuing to

be able to be handled by the observer without danger for himself or to the organs.

CONCLUSIONS

Glycerol impregnation technique of dissected organs is an effective process of tissue conservation, low time consuming and inexpensive, while maintaining the relationship of the anatomical elements among themselves. Additionally the natural color of the various tissues is almost unchanged. Two other major advantages are its pliability and long term resistance, enabling its handling without damaging it. It is our belief that this preservation technique of macroscopic anatomical parts is of high interest to undergraduates and postgraduate students, as you can see from the photos included in this work.

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VARIANTES ANATÓMICAS DOS MÚSCULOS *EXTENSOR DIGITORUM LONGUS* E *EXTENSOR DIGITORUM BREVIS*

ANATOMICAL VARIATIONS OF *EXTENSOR DIGITORUM LONGUS* AND *EXTENSOR DIGITORUM BREVIS* MUSCLES

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RESUMO

Introdução: Estão documentadas variações do padrão dos tendões dos músculos extensores dos dedos do pé.

Materiais e métodos: Foi realizada a dissecação das regiões anterior da perna e dorsal do pé de dois cadáveres.

Resultados: Identificaram-se dois casos de variações. No Caso 1, o músculo *Extensor digitorum brevis* originava cinco tendões, dois dos quais se inseriam no *hallux*. Os restantes três tendões inseriam-se no segundo, terceiro e quarto dedos. No Caso 2, o músculo *Extensor digitorum longus* originava 3 tendões: o tendão medial dividia-se em dois feixes, um para o segundo dedo e outro para o terceiro dedo; o tendão intermédio inseria-se no quarto dedo; o tendão lateral inseria-se no quinto dedo. **Conclusão:** O conhecimento das variações destes músculos é importante na prática clínica.

Palavras-chave: Anatomia, *Extensor digitorum longus*, *Extensor digitorum brevis*

ABSTRACT

Introduction: Variation patterns of extensor tendons of the foot have been documented. **Materials and methods:** The anterior region of the leg and dorsal region of the foot were dissected in two cadavers. **Results:**

Two cases of variations were identified. In Case 1 the *Extensor digitorum brevis* originated five tendons, two of which were inserted in the *hallux*. The other tendons were inserted in the second, third and fourth toes. In Case 2 the *Extensor digitorum longus* originated three tendons: the medial tendon sent one slip to the second toe and another to the third toe; the intermediate tendon was inserted in the fourth toe and the lateral tendon was inserted in the fifth toe. **Conclusion:** In clinical practice, the variations of these muscles should be taken into account.

Keywords: Anatomy, *Extensor digitorum longus*, *Extensor digitorum brevis*

INTRODUCTION

The morphology of *Extensor digitorum longus* and *Extensor digitorum brevis* is subject of variations. Several studies using cadavers report that those muscles don't always meet the most common pattern that is described in Anatomy textbooks.^{3, 13, 15, 16, 17}

Their variations must be documented once this muscles may be used as free flaps or tendon grafts in the repair of ligaments and tendons of the hand and foot in plastic and orthopaedic surgeries.

We report two variations in the pattern of the tendons of *Extensor digitorum longus* and *Extensor digitorum brevis* found during dissection of the inferior limbs of two cadavers.

MATERIAL AND METHODS

The dissection of the anterior region of the leg and the dorsal region of the foot was performed in two formalin-embalmed cadavers, one female age 61 (Case 1) and one male age 65 (Case 2), both Caucasians. The dissection work took place at Institute of Normal Anatomy, Faculty of Medicine, University of Coimbra. The muscles in those regions were isolated from their proximal origin. Their tendons were identified and isolated until their insertion in the toes. The variations found were documented and photographed.

RESULTS

During dissection of the anterior region of the leg and dorsal region of the foot, two cases of variations in the pattern of *Extensor digitorum brevis* and *Extensor digitorum longus*'s tendons were found (Cases 1 and 2).

In Case 1 (right foot) the *Extensor digitorum brevis* muscle was organized in five muscular slips

that ended in five tendons. The two medial tendons were inserted in the dorsal surface of the proximal phalanx of the hallux. The other three tendons were attached to the tendons of *Extensor digitorum longus* for the second, third and fourth toes (Figure 1).

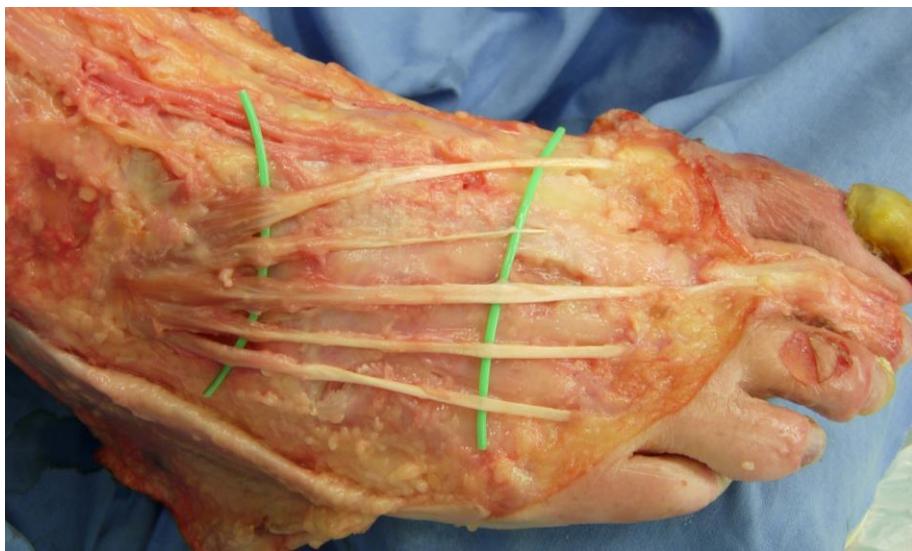


Fig.1: Case 1 – EDB: *Extensor digitorum brevis*. EDB originates 5 tendons. T1 and T2: tendons for the hallux; T3: tendon for 2nd finger; T4: tendon for 3rd finger; T5: tendon for 4th finger.

In Case 2 (left leg) the *Extensor digitorum longus* originated three tendons, after crossing the *extensor retinaculum*. The medial tendon was divided in the dorsum of the foot in two slips: one slip was inserted in the second toe whilst the other was

inserted in the third toe. The intermediate tendon was inserted in the fourth toe and the lateral tendon was inserted in the fifth toe (Figure 2).

No variations were found in the contralateral limbs of both cadavers.

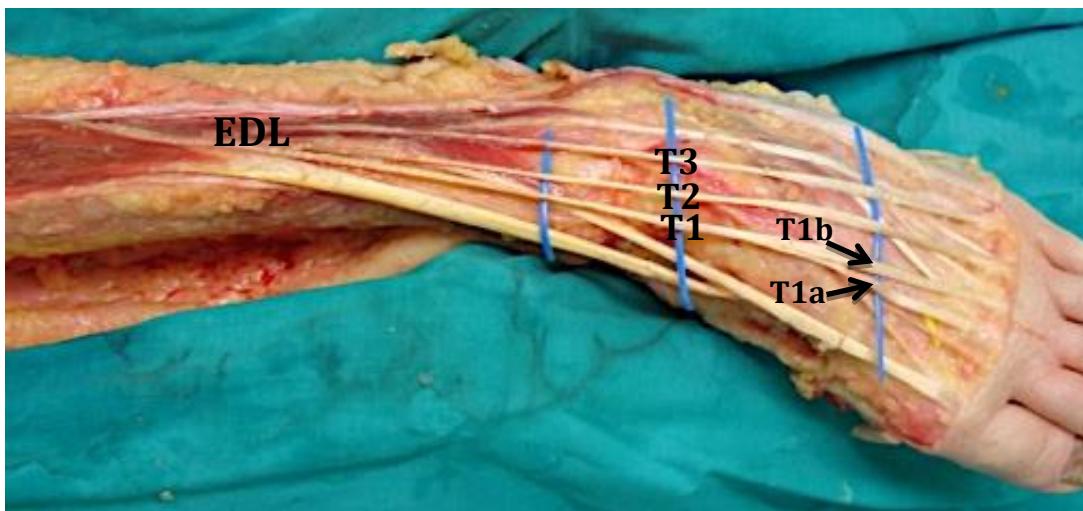


Fig. 2: Case 2 – EDL: *Extensor digitorum longus*. EDL originates 3 tendons. T1: medial tendon: divides into T1a: tendon for 2nd finger and T1b: tendon for 3rd finger; T2: tendon for 4th finger; T3: tendon for 5th finger.

DISCUSSION

During dissection of the anterior region of the leg and dorsal region of the foot of two

cadavers, two variations were found in the arrangements of the tendons of the extensor muscles of the foot.

The *Extensor digitorum brevis* is located in the dorsal region of the foot. Its muscular fibres are usually organized in four muscular bundles that originate four tendons: the medial tendon is inserted in the proximal phalanx of the *hallux* and acts as extensor of the metatarsophalangeal joint; the other three tendons are usually attached to the tendons of *Extensor digitorum longus* to the second, third and fourth toes and assist in extending the phalanges of the middle three toes via the tendons of *Extensor digitorum longus*.^{5,7,14,18}

In Case 1, the *Extensor digitorum brevis* originated five tendons. The two medial tendons were inserted in the *hallux*. Several variations of this muscle have been described in literature, such as the existence of accessory bundles to talus or navicular bones and extra tendons for the fifth toe.¹⁸ There have been reported cases of absence of some of its tendons or even the whole muscle.^{17,18} It was described that additional musculotendinous units from *Extensor digitorum brevis* may be present in about 72,5% of cases.⁷ This work describes one extra tendon from the *Extensor digitorum brevis* muscle to the *hallux* extensor apparatus. However, supernumerary extensor tendons to the *hallux* have often been described.³

The *Extensor digitorum longus* is located in the anterior compartment of the leg. Its fibres usually originate one tendon that divides into four slips that are attached to the four lateral toes of the foot. This muscle is an extensor of the last four toes of the foot.^{5,7,11,14,18}

In Case 2, the *Extensor digitorum longus* originated three tendons in the inferior third of the

leg. After crossing the *Extensor retinaculum*, at the dorsum of the foot, the medial tendon divided in two slips for the third and fourth toes. In the literature some variations have been reported concerning this muscle's distal insertion: there may be double tendons to the second and fifth toes; accessory slips to the *hallux* or metatarsals may be present; accessory slips that are attached to the interosseous muscles have also been reported.^{15,18,16} The absence of some of its tendons is very rare.¹⁵ This work describes a late division of the medial tendon of the *Extensor digitorum longus* muscle in the dorsum of the foot.

Knowledge about the variants of extensor muscles of the foot is important for clinical practice. The *Extensor digitorum brevis* may be used as free or pedicled flap in the repair of defects of soft tissues in the ankle and foot.^{2,4,9,10,12,20} The tendons of *Extensor digitorum longus* may be used for reconstruction procedures in the hand, calcaneal tendon and lateral ligaments of ankle joints.^{1,6,8,15,19} It is important to be aware of the possible variants of the arrangements of these muscles's tendons while harvesting the tendon grafts in plastic or orthopedic procedures.

CONCLUSIONS

The variations observed in this study were found during routine dissection of two cadavers. Knowledge of the morphology and variations of the extensor muscles of the foot is important for surgeons, once these muscles may be used for multiple procedures.

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Músculo esquelético estriado em atletas federados através da avaliação da intensidade de sinal por Ressonância Magnética

Striated skeletal muscle in elite athletes through assessment of signal by Magnetic Resonance Imaging

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Resumo

Objetivo: Analisar a diferença entre a intensidade média do sinal obtido por Ressonância Magnética (RM) nos músculos de maior volume nos atletas federados em futebol comparativamente aos indivíduos não praticantes de desporto. **Material e Métodos:** Realizou-se uma recolha retrospectiva de 30 exames de RM agrupados em duas sub-amostras: uma com 15 exames de atletas federados em futebol e outra com 15 exames pertencentes a indivíduos sedentários (grupo de controlo). Foram analisadas imagens ponderadas em Densidade Protónica (DP) e em *Water Fat-Separation T2** (WFS T2*) nos músculos semimembranoso, gastrocnémio lateral e vasto medial (joelho); vasto crural e reto femoral (coxa); e solear (tibio-társica). Para a avaliação do deltóide (ombro) foram usadas as ponderações T1 e WFS T2*. As imagens foram processadas e avaliadas no programa RadiAnt DICOM Viewer™ e medidas por dois observadores independentes através da colocação de ROI (*Region of Interest*) com uma área de 0,6 cm². **Resultados:** Nas imagens ponderadas em DP os atletas apresentaram uma intensidade média de sinal superior (10690,17) relativamente aos indivíduos sedentários (6468,97). Pelo contrário, na sequência com saturação do sinal da gordura WFS T2*, os atletas apresentaram uma intensidade

de sinal inferior (9085,04) contra (12502,48). Pelo teste de τ -*Kendall* obteve-se uma elevada medida de concordância entre os observadores ($\tau=0,855$). **Conclusões:** No conjunto das ponderações verificou-se haver um acréscimo global de sinal nos atletas relativamente aos indivíduos sedentários podendo este ser atribuído a edema fibrilar associado ao exercício físico. É possível estimar a atividade desportiva controlada através da quantificação muscular por RM estrutural.

Palavras-chave: Ressonância Magnética, Atividade desportiva controlada, Músculos, Intensidade de Sinal.

Abstract

Objective: To analyze if there are statistically significant differences between the average signal intensity by MRI on larger volume muscle in elite football athletes compared to subjects not practicing sports.

Materials and Methods: Was performed a retrospective data collection into two nonprobabilistic sub-samples: 15 cases belonging to elite athletes in football and another one with 15 cases belong to sedentary individuals (control group). In each case, were selected images obtained by Proton Density (PD) and Water Fat-Separation T2* (WFS T2*) sequences. The studied muscles were the *semimembranosus*, *gastrocnemius* and *vastus intermedius* (knee), *vastus crural* and *rectus femoris* (thigh), *soleus* (tibiotarsal joint) and deltoid (shoulder).

The images were evaluated by application RadiAnt DICOM Viewer™ and measured by two independent observers by placing ROI(s) (*Region of Interest*) with an area of 0,6 cm². Results: On the PD sequence, the athletes had a higher average signal intensity (10690,17) than the sedentary individuals (6468,97). On the other hand, on the WFS T2* sequence the athletes showed a lower signal intensity (9085, 04) against (12502, 48).

The *Kendall* τ test yielded a high measure of agreement between observers ($\tau=0,855$).

Conclusions: It is concluded that there is an increase of signal intensity in elite athletes relatively to sedentary subjects that may be assigned to a swelling of the muscle fibers associated with physical activity.

The controlled physical activity can be measured through the quantification of the muscle by structural MRI.

Keywords: Magnetic Resonance, Controlled sporting activity, Muscles, Signal Intensity.

Introdução

O músculo esquelético com o tecido conjuntivo associado compreende cerca de 40% do peso corporal, sendo responsável por diversas funções, tais como locomoção e postura.¹ Este tipo de musculatura está sujeita a variadas lesões como por exemplo estiramentos e contusões.² Para avaliação destas lesões, têm sido amplamente utilizados os métodos de imagem tais como a Ultrassonografia e a Ressonância Magnética (RM).³ A RM oferece uma avaliação anatómica e patológica pormenorizada das lesões musculares, uma vez que as imagens multiplanares facilitam o estudo da anatomia, da localização e da definição da intensidade de sinal das lesões.⁴

Músculo esquelético

O sistema músculo-esquelético é o mais volumoso sistema orgânico do ser humano. Dele fazem parte os músculos estriados cujas funções dependem, na sua maioria, do controlo voluntário ou consciente pelo sistema nervoso.¹

A unidade básica de organização histológica do músculo esquelético é a fibra muscular, uma célula larga, cilíndrica e polinucleada. As fibras musculares agrupam-se em fascículos que por sua vez se associam para formar fibrilhas e feixes, culminando nos diferentes tipos de músculos.⁵

As lesões musculares apresentam rotura de fibras, na junção músculo-tendinosa, no tendão ou na inserção óssea. Os sinais e sintomas deste tipo de lesões são variados, mas incluem o edema, a dor, a perda da função muscular e a inflamação.⁶

A inflamação é a resposta dos tecidos à lesão, caracterizando-se pela movimentação de fluídos, de proteínas plasmáticas e de leucócitos, em direção ao tecido afetado. A resposta inflamatória aguda é considerada como a explicação para a sensação de dor muscular cerca de 24 a 48 horas após o exercício físico. No tecido muscular lesado os monócitos tornam-se macrófagos e são responsáveis pela remoção do tecido necrótico. Os macrófagos libertam os mediadores da inflamação - prostaglandinas, que por sua vez, sensibilizam os

recetores locais da dor, intensificando a estimulação dolorosa.⁷

O edema justifica-se através da ineficácia da ATPase transportadora de sódio e potássio (Na^+/K^+) levando a uma crescente concentração de Na^+ no interior da fibra muscular, e consequentemente ao acumular de líquido no espaço intersticial. Conjuntamente verifica-se uma saída de K^+ com diminuição do *pH* intracelular.⁸

A perda da função muscular pode resultar da rotura total das fibras musculares após a realização de exercício físico intenso, ou seja existe hipersensibilidade no ponto de rotura e a contração da massa muscular próxima do foco de lesão.⁵ O processo inflamatório gerado aumenta à medida que ocorrem micro roturas das fibras musculares.⁹ Esta lesão induz uma resposta inflamatória com a migração de células e libertação de substâncias que promovem a remoção dos tecidos lesados e estimulam, desta forma, o processo de regeneração.⁵

Implicações da prática de exercício físico

O futebol é um dos desportos que merece grande destaque devido à sua elevada popularidade e pelo

facto de ser responsável por cerca de 50 a 60% de todas as lesões musculares desportivas. Estas lesões têm-se tornado um desafio constante devido ao tempo de recuperação que afasta o atleta dos treinos e competições.¹⁰ As lesões decorrentes deste desporto podem ocorrer em diversos locais do corpo. Cerca de 68-88% das lesões ocorrem na articulação do joelho e na região da articulação tibio-társica.¹¹ No futebol verificou-se que em 281 jogadores avaliados, a grande maioria das lesões se localizou no membro inferior. Cerca de 31,65% das lesões ocorreram no joelho; 23,38% na articulação tibio-társica; 16,91% na coxa; 8,99% no pé e 5,75% na perna.¹²

As distensões musculares, principalmente do quadrípede e dos músculos posteriores da coxa, são as lesões atléticas mais comuns.¹³

O joelho é uma das áreas anatómicas mais propensa a lesões devido à sua mobilidade e à variedade de tensões a que é submetido.¹⁴ Os músculos mais comumente afetados são os isquiotibiais, quadrípedes e gastrocnépios, músculos estes bi-articulares que estão sujeitos a forças de aceleração e desaceleração.¹⁵

A articulação tibio-társica, por sua vez, permite os movimentos entre a perna e o pé, sendo das articulações do corpo a que mais facilmente sofre traumatismos. A lesão mais frequente é condicionada pelos movimentos combinados de adução, rotação interna e extensão dorsal do pé sobre a perna e donde resulta o estiramento parcial ou total dos ligamentos externos.¹⁶ Outra área anatómica frequentemente sede de lesões nos desportos é o ombro representando entre 8 a 13% de todas as lesões desportivas.¹⁷ A função coordenada dos músculos do ombro é essencial para o movimento eficiente desta articulação e o músculo deltóide evidencia-se por ser motor primário do movimento de abdução.¹⁸ Este músculo pode apresentar lesões que tornam difícil e dolorosa a movimentação do membro superior.¹⁹

As lesões desportivas podem ser causadas por fatores intrínsecos como a *performance* muscular e por fatores extrínsecos como o local do treino, o equipamento usado e as condições ambientais.^{15,20}

Os fatores de risco para a existência de lesões no futebol são as assimetrias entre o membro

dominante e o não dominante; a repetição crónica de alguns movimentos que podem afetar a integridade dos membros; movimentos de aceleração e desaceleração dos segmentos proximais e distais e o impacto que pode comprometer o alinhamento estático do membro inferior a longo prazo.^{10,12,15,20}

O diagnóstico precoce e o prognóstico são informações importantes tanto para o atleta que possui a lesão como para o clube, uma vez que podem inviabilizar os treinos e as competições levando desta forma a perdas financeiras e competitivas.^{10,15} Pensa-se que a RM com a sua elevada sensibilidade poderá visualizar alterações subtis induzidas pela prática de exercício físico, nos tecidos musculares.²¹

O exercício físico produz alterações na quantidade e distribuição de água no sistema músculo esquelético e a RM, sendo sensível a estas alterações, pode detetar essas modificações em condições de imagem apropriadas. O exercício físico aumenta o teor total de água no músculo, principalmente no espaço extracelular, enquanto

um esforço mais intenso leva a incrementos de água e sódio no espaço intracelular.²²

Pelas suas potencialidades no estudo de partes moles, a RM tem-se demonstrado um teste de diagnóstico de excelência, nos exames do sistema músculo-esquelético, sendo cada vez mais solicitada, de que são exemplo as estatísticas das Olimpíadas de Londres, 2012 onde o exame de RM foi, de entre todos os métodos de imagem, o mais realizado aos atletas, perfazendo

49% de todos os exames efetuados. O mesmo se verificou nos exames realizados nos Jogos Paralímpicos de 2012 onde cerca de 39% dos exames são desta modalidade. As mesmas estatísticas referem ainda que joelho, coluna e articulação tibio-társica foram as áreas anatómicas que com maior frequência foram submetidas a exames de diagnóstico por imagem.²³

Princípios Físicos da Ressonância Magnética

A RM é um método de aquisição de imagem estabelecida na prática clínica e em crescente desenvolvimento.²⁴ Este método de imagem permite uma completa avaliação das lesões musculares,

devido à aplicação das imagens multiplanares no estudo da anatomia, da localização e da definição da intensidade de sinal das lesões através das ponderações T1 e T2. Deste modo, proporciona uma caracterização tecidual que permite o perfeito diagnóstico, a graduação quanto à gravidade das diversas lesões e indicações de prognóstico.⁴ A intensidade de sinal em RM é devida à leitura da magnetização transversa representada na imagem com um dado valor de intensidade de pixel.²⁵

As ponderações básicas T1 e T2 são sequências que permitem produzir e contrastar as diferenças T1 e T2 de cada tecido.²⁶ A imagem por ponderação DP é representada pela diferença do número de protões por unidade de volume, sendo este o fator determinante na formação do contraste da imagem. Para se alcançar a imagem em ponderação DP, os efeitos T1 e T2 devem ser suprimidos.²⁷ Esta sequência é útil para avaliação da anatomia das estruturas e ainda das alterações patológicas.²⁸

Outro fator a ter em conta na produção de contraste é a intensidade do campo magnético que influencia por exemplo os protões no tecido adiposo os quais entram em ressonância em frequências próximas à

da água.²⁹ Para tal utilizam-se técnicas de supressão de gordura, como o *Fat-Saturation* que é usado para minimizar o sinal da gordura relativamente aos tecidos circundantes, sendo bastante utilizado em RM músculo esquelética para realçar o sinal de edema.³⁰ Em alternativa emprega-se a técnica WFS T2*, para se obterem imagens da água e da gordura em diferentes frequências de ressonância evidenciando seletivamente as imagens com sinal de água ou com sinal de gordura. Empregando esta técnica de combinação linear é também possível obter imagens com um realce das estruturas líquidas e cartilagíneas pelo apagamento do sinal da gordura.³¹⁻³⁴

Pretendeu-se com este estudo demonstrar se existem diferenças estatisticamente significativas entre o sinal médio de RM dos músculos de maior volume nos atletas federados em futebol comparativamente aos indivíduos não praticantes de desporto.

Materiais e Métodos

O estudo tem um caráter exploratório, observacional e comparativo com uma recolha

retrospectiva de dados. A amostra consta de N=30 casos divididos em duas sub-amostras de 15 casos cuja seleção foi não probabilística. Foram selecionados 30 exames de RM, sendo 15 de atletas federados em futebol (casos) e 15 de indivíduos não praticantes de desporto ou sedentários (grupo de controlo). Os atletas federados foram na sua totalidade do sexo masculino, com idades compreendidas entre os 22 e os 40 anos de idade ($\mu=28,5$; ($Md=28$). Quanto aos casos de controlo, 5 indivíduos foram do sexo feminino e 10 do sexo masculino, com idades compreendidas entre os 17 e os 62 anos de idade ($\mu=32,1$); ($Md=29$). Para responder aos princípios éticos e deontológicos, foram observadas as orientações dos responsáveis clínicos da instituição e respeitado o anonimato dos participantes.

Os exames foram obtidos a partir dum equipamento de Ressonância Magnética marca Hitachi® modelo *Airis Elite* de campo aberto de 0,30 Tesla e as imagens visualizadas em formato de imagem DICOM (*Digital Imaging and Communication in Medicine*). Por cada

exame a analisar foram selecionadas duas imagens, uma da sequência DP ou T1 (Tabela

1) e outra da sequência WFS T2* (Tabela 2).

Tabela 1 – Protocolo utilizado na sequência DP e T1 para o ombro.

	FOV (mm)	TR (ms)	TE (ms)	FA (graus)	Slices (Nº)	TH (mm)	GAP (mm)	Matriz	Fase	Freq.
Joelho	200	1320	20	90°	20	4,0	4,5	512	172	288
Coxa	320	1320	20	90°	20	6,0	6,5	512	224	288
Tibio-társica	200	1188	20	90°	18	3,5	4,5	256	192	388
Ombro	240	468	20	90°	12	4,0	5,0	512	200	260

Legenda: *FOV* (Campo de visão); *TR* (Tempo de repetição de sequência); *TE* (Tempo de leitura de eco); *FA* (Ângulo de báscula); *Slices* (Planos); *TH* (Espessura de Corte); *GAP* (Intervalo entre planos); Matriz (linhas por colunas); Fase (Fase) e Freq. (Frequência).

Tabela 2 – Protocolo utilizado na sequência WFS T2*.

	FOV (mm)	TR (ms)	TE (ms)	FA (graus)	Slices (Nº)	TH (mm)	GAP (mm)	Matriz	Fase	Freq.
Joelho	220	742	17,1	30°	20	4,0	5,0	256	172	256
Coxa	320	556	17,1	30°	15	6,0	6,6	512	192	256
Tibio-társica	300	360	25	90°	16	3,0	4,0	256	172	320
Ombro	250	444	20	30°	12	4,0	5,0	256	172	224

Legenda: *FOV* (Campo de visão); *TR* (Tempo de repetição de sequência); *TE* (Tempo de leitura de eco); *FA* (Ângulo de báscula); *Slices* (Planos); *TH* (Espessura de Corte); *GAP* (Intervalo entre planos); Matriz (linhas por colunas); Fase (Fase) e Freq. (Frequência).

Nos exames de ombro avaliaram-se imagens da sequência T1 e WFS T2*. Tal escolha deveuse a uma opção da equipa clínica na fase de aquisição de imagens por esta apresentar, contrariamente às outras regiões anatómicas, maior especificidade para o estudo morfológico da área em apreciação.

Esta opção tem ainda fundamento nas características técnicas do equipamento em uso.

A sequência DP permite uma boa avaliação de áreas anatómicas que envolvem muitas das estruturas ligamentares, devido à presença de mioblastos e do tecido conjuntivo de suporte.³⁵ Foram estudados grupos musculares em quatro áreas anatómicas e identificadas as respetivas sequências e planos:

As imagens pertencem ao grupo dos casos – atletas.

joelho (DP Sagital e WFS T2*Sagital), coxa (DP Sagital e WFS T2*
Coronal), tibio-társica (DP Coronal e WFS T2*
Sagital) e ombro (T1 Coronal e WFS T2* Coronal). No joelho foram analisados três músculos: semimembranoso, gastrocnémio lateral e vasto medial. Na coxa foram avaliados o vasto crural e reto femoral. Na região da articulação tibio-társica foi examinado o músculo solear e por fim, no ombro o músculo deltóide. Fig.1

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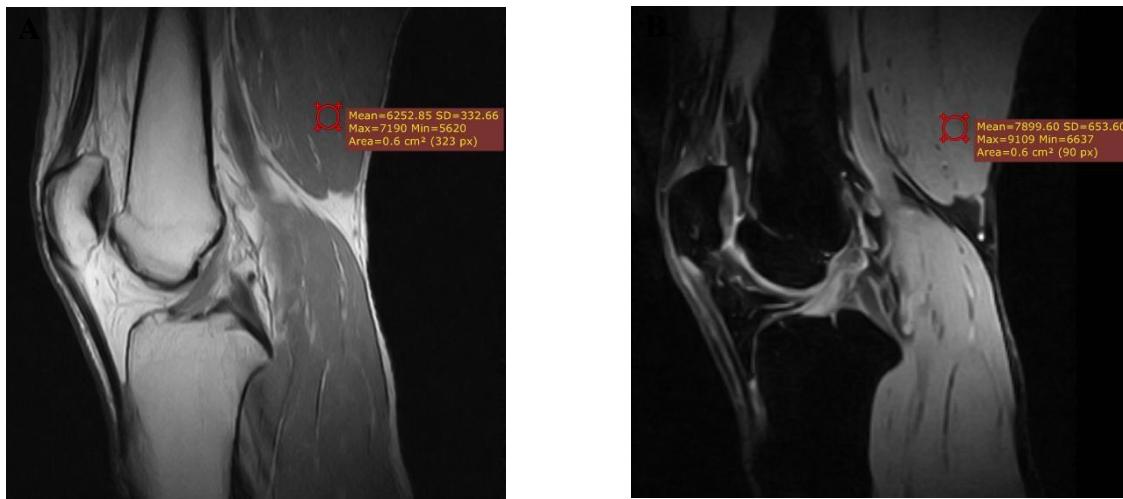


Figura 1 – Imagens representativas da metodologia de medida – local e área da ROI no músculo semimembranoso, no joelho, obtidas em plano sagital na ponderação DP (A) e no mesmo plano na ponderação WFS T2* (B).

Foi feita uma seleção amostral não probabilística, sequencial, por tipicidade (para os casos), entre janeiro de 2012 e janeiro de 2013. Foram determinados os critérios de exclusão inerentes às *i*) contraindicações para estudo por RM e também *ii*) ter história de intervenção cirúrgica na área anatómica em estudo, *iii*) ter sofrido traumatismo recente (menos de 1 mês), *iv*) ter idade inferior a 16 anos e *v*) obedecer ao mesmo protocolo de aquisição. Esta metodologia de seleção foi aplicada aos sujeitos que se apresentaram na instituição para controlo de patologia menisco-ligamentar.

A aquisição, processamento, validação, seleção, observação e análise das imagens foram efetuados por *experts* em Radiologia e em RM músculo-esquelética e treino em qRM – RM de quantificação. A seleção das imagens, dos músculos a analisar bem como da orientação espacial eleita obedeceu ao critério de melhor evidência e maior volume da estrutura anatómica alvo. Atendendo à reproduzibilidade, para todos os sujeitos e ponderações, as imagens a avaliar coincidiram com o centro da sequência com um desvio de (± 1) imagem mantendo-se o critério para todas as

avaliações. A observação respeitou iguais requisitos de tempo, resolução de ecrã e luminosidade.

A medição da intensidade de sinal nas imagens obtidas foi realizada através do programa

RadiAnt DICOM ViewerTM.³⁶

Foram estabelecidos critérios de área e localização para a colocação das ROI(s) (*Region of Interest*).²¹

Estas foram limitadas a uma área de 0,6 cm² e como atributos para a análise das imagens: *i*) ser colocadas na área de maior volume muscular; *ii*) evitar sempre a periferia da estrutura; *iii*) evitar estruturas vasculares, tendinosas e gordura; e *iv*) colocar a ROI o mais central no FOV (*Field of View*) que comprehende a área anatómica para evitar

perdas de sinal e artefacto de “fora de campo” e *v*) equivalente proximidade à zona sensível da bobina.

O tratamento dos dados foi realizado com recurso ao programa estatístico SPSS® (*Statistic Package for Social Sciences*) versão 19 para Windows e o nível de significância foi de $\alpha=5\%$ e considerados significativos os valores de $p <0,05$. Procedeu-se à validação do pressuposto da normalidade da distribuição da amostra recorrendo aos testes de

ajustamento: *KolmogorovSmirnov* e *Shapiro-Wilk*. Tendo em conta a amostragem e o músculo, aplicou-se o teste *t* de *student* para comparação das médias de duas amostras independentes, nos músculos gastrocnémio lateral, vasto medial, reto femoral e solear. Nas situações em que houve violação do pressuposto da normalidade adotou-se a alternativa não paramétrica - Teste

Mann-Whitney U.

Foram calculadas as diferenças de intensidade de sinal (apresentadas em unidades arbitrárias), consoante a amostragem e o músculo; a sequência e o músculo; e a amostragem e a sequência.

Resultados

Foram avaliadas um total de 60 imagens de atletas federados em futebol e de indivíduos sedentários por dois observadores independentes. Obteve-se uma medida de concordância de $\tau=0,855$ entre os observadores, ou seja, existiu elevada concordância entre o observador 1 e 2.

Verificaram-se diferenças estatisticamente significativas nos músculos semimembranoso e

solear ($p=0,014$ e $p=0,036$) respetivamente (Tabela 3).

Tabela 3 – Diferenças obtidas da intensidade de sinal consoante a amostragem e o músculo.

	Atleta		Indivíduos sedentários			
	Média	Desvio Padrão	Média	Desvio Padrão	Teste	P
Semimembranoso	4169,59	3065,75	9380,47	4358,84	<i>Mann-Whitney U</i>	0,014
Gastrocnémio lateral	5964,85	4549,19	8693,41	6938,07	<i>t student</i> duas amostras	0,267
Vasto medial	8441,84	668,92	7110,77	3890,08	<i>t student</i> duas amostras	0,546
Vasto crural	23090,64	36855,17	6905,73	1379,89	<i>Mann-Whitney U</i>	0,065
Reto femoral	13281,69	1212,65	12172,89	3551,78	<i>t student</i> duas amostras	0,254
Solear	7623,79	2086,08	10754,59	3291,97	<i>t student</i> duas amostras	0,036
Deltóide	10738,34	4436,14	10643,53	8428,63	<i>Mann-Whitney U</i>	0,442

No músculo semimembranoso, os indivíduos sedentários apresentaram uma intensidade média de sinal de 9380,47 e os atletas apresentaram de 4169,59. No músculo solear, por sua vez, os indivíduos sedentários demonstraram uma intensidade média de sinal de 10754,59 e os atletas de 7623,79.

Consoante a sequência e o músculo foram utilizadas estatísticas não-paramétricas, teste *Mann-Whitney U*, para os músculos semimembranoso e

gastrocnémio lateral. O músculo reto femoral e o deltóide apresentaram diferenças significativas ($p=0,03$ e $p=0,00$ respetivamente) consoante a sequência aplicada (DP ou WFS T2*) (Tabela 4). No músculo deltóide a intensidade média de sinal, na sequência WFS T2* foi 16013,42 e no músculo reto femoral de 14472,31 enquanto que para os mesmos músculos a ponderação em DP apresentou valores de 5368,46 e 10982,28.

Tabela 4 – Diferenças da intensidade de sinal consoante a sequência e o músculo.

	DP		WFS T2*			
	Média	Desvio Padrão	Média	Desvio Padrão	Teste	P
Semimembranoso	5039,55	2446,77	8510,51	5536,35	<i>Mann-Whitney U</i>	0,060
Gastrocnémio lateral	5704,82	4204,83	8953,44	7032,47	<i>Mann-Whitney U</i>	0,242
Vasto medial	6264,21	2922,64	9288,41	1456,82	<i>t student</i> duas amostras	0,113
Vasto crural	19923,23	38066,07	10073,13	2329,28	<i>t student</i> duas amostras	0,477
Reto femoral	10982,28	2478,04	14472,31	1298,97	<i>t student</i> duas amostras	0,030
Solear	8824,17	1716,09	9554,21	4188,13	<i>t student</i> duas amostras	0,655
Deltóide	5368,46	1528,59	16013,42	4861,91	<i>t student</i> duas amostras	0,000

Quanto às diferenças da intensidade de sinal por sequência estudada, os atletas possuem imagens de RM com uma intensidade de sinal superior aos indivíduos sedentários (9887,60 e 9485,73 respectivamente). Pelos dados da sequência DP os atletas também possuem uma intensidade de sinal

superior (10690,17), porém, no que diz respeito à sequência WFS T2* a relação inverte-se uma vez que os indivíduos sedentários possuem uma intensidade de sinal superior (12502,48 vs 9084,04). Estas diferenças revelaram-se estatisticamente significativas com $p=0,012$ (Tabela 5).

Tabela 5 – Diferenças da intensidade de sinal consoante a amostragem e a sequência.

		Média	Desvio Padrão	P
DP	Atletas	10690,17	19855,79	0,254
	Indivíduos sedentários	6468,97	2649,08	
WFS T2*	Atletas	9085,04	4665,89	0,012
	Indivíduos sedentários	12502,48	5489,72	
Global	Atletas	9887,60	14343,18	0,839
	Indivíduos sedentários	9485,73	5245,71	

Discussão

O futebol caracteriza-se por uma elevada exigência física determinado por uma série de movimentos que poderão provocar lesões aos praticantes desta modalidade e variações do metabolismo. As lesões musculares encontram-se entre as mais comuns nos atletas federados em futebol.^{37,38} O diagnóstico precoce destas lesões é uma informação crucial tanto para o atleta que possui a lesão como para o clube, uma vez que pode inviabilizar os treinos e as competições levando desta forma a custos acrescidos.^{10,15} Assim, é essencial colmatar lacunas existentes no diagnóstico destas lesões e que possam facilitar e proporcionar uma metodologia de validação fiável. A RM é o exame de diagnóstico radiológico que permite uma melhor avaliação destas lesões, visto fornecer imagens multiplanares que facilitam o estudo tanto da anatomia como da localização e definição da lesão em causa.⁴

Tendo em conta que as mudanças induzidas pelo exercício físico condicionam um aporte de água entre compartimentos celulares, sensivelmente ~7% no compartimento intracelular e ~40% no espaço extracelular, é de se esperar que exista um

aumento da hiperintensidade nas imagens de RM, visto que esta é sensível às alterações da água. Alguns autores sugerem que este facto possa ser o resultado de uma reorganização do líquido intracelular durante o exercício físico.³⁹ Com a realização de exercício físico intenso a componente T2 de RM aumenta entre 10 a 30% e consequentemente os músculos aparecem mais hiperintensos nesta ponderação do que em condições normais. Especula-se que estas mudanças na componente T2 sejam resultados do TE utilizado (TE longo > 80 ms ou TE curto – 20 a 40 ms).³⁹ Desta forma, a intensidade de sinal pode ser utilizada para distinguir quais os músculos ativados em diferentes fases motoras.²¹ Tal poderá resultar do facto dos sujeitos terem sido submetidos a uma carga intensa de exercício físico imediatamente antes de realizarem o exame de RM.

É também sugerido que estas mudanças de intensidade de sinal nos músculos possam estar associadas a uma diminuição do *pH* que pode durar até várias horas após o exercício físico.⁴⁰ Os músculos em recuperação do exercício físico têm uma alteração química na água, comparativamente

com fibras musculares normais e estas mudanças podem durar até 12 horas após a realização do exercício.⁴¹

Na sequência WFS T2* os indivíduos sedentários possuem uma média de intensidade de sinal (12502,48) mais elevada do que os atletas federados em futebol (9085,04). Tal poderá ser explicado pelo facto de ser uma sequência com saturação do sinal de gordura ou pelos baixos valores de TE utilizados. Pode-se ainda argumentar a utilização desta modalidade de saturação de gordura ser obtida pelo método de seleção de frequências sendo que a componente T2 dos tecidos, no que diz respeito à água, possa estar atenuada. Para clarificar esta *nuance* também se podem utilizar os métodos de pulso de inversão para saturação da gordura. O método de saturação espectral aqui aplicado poderá ter influenciado o sinal da água pela seleção pouco precisa do pico de frequência da água/gordura.

O valor do sinal dos músculos analisados que vão de encontro à bibliografia apresentada são o vasto medial (Atletas = 8441,84 vs Indivíduos sedentários = 7110,77); vasto crural (Atletas = 23090,64 vs

Indivíduos sedentários = 6905,73); reto femoral (Atletas = 13281,69 vs Indivíduos sedentários = 12172,89) e deltóide (Atletas = 10738,34 vs Indivíduos sedentários = 10643,53), onde os músculos dos atletas federados apresentam uma intensidade de sinal superior comparativamente aos indivíduos sedentários. (Tabela 3)

Relativamente às diferenças de intensidade de sinal consoante o músculo e atendendo à sequência verifica-se que todos os músculos analisados, exceto o vasto crural, possuem uma maior intensidade de sinal na sequência WFS T2*. Globalmente, a sequência DP possui uma média de intensidade de sinal superior nos atletas federados (10690,17) comparativamente como os indivíduos sedentários (6468,97). Assim sendo, pode-se afirmar que, nesta sequência, se verifica um acréscimo na intensidade de sinal nos atletas federados em futebol relativamente aos indivíduos sedentários. (Tabela 5)

Conclusões

A atividade desportiva tem um grande impacto cultural assim como financeiro. A RM devido à sua elevada especificidade e sensibilidade para estruturas de tecidos moles oferece uma avaliação pormenorizada e proporciona uma completa caracterização tecidual.

Nos estudos realizados no âmbito da RM enquadrada no desporto federado, considera-se este método de diagnóstico por imagem estrutural muito importante e útil. Outros estudos têm selecionado abordagens metodológicas diferentes tal como a RM funcional nomeadamente a técnica BOLD (*Blood Oxigen Level Dependent*) utilizando o sangue como biomarcador e utilizando as diferentes propriedades magnéticas da oxi-hemoglobina e da desoxihemoglobina, ou a técnica DTI (*Diffusion Tensor Imaging*) para avaliar a transferência de água entre compartimentos celulares, porém estas técnicas além de serem demoradas, exigem equipamentos de ultra alto campo e gradientes multidireccionais e não estão disponíveis na maioria dos equipamentos existentes em Portugal. Para além disso, a abordagem por RM de caraterização

estrutural, pelas suas potencialidades, deve ser objeto de exploração uma vez que não se encontra exaustivamente estudada.

Como limitações deste estudo consideram-se as diferenças de idades entre os grupos, a diferença de género, a dimensão da amostra e o facto da avaliação por RM não ter ocorrido no imediato após prática de atividade desportiva.

Respondendo ao objetivo do estudo conclui-se que a RM estrutural permite medir as alterações musculares produzidas pela atividade física através da medida da intensidade de sinal de pixel. Observou-se haver um aumento da intensidade média global de sinal no exame de RM nos atletas federados sobre os sedentários (9887,60; 9485,73 respetivamente), se bem que esse aumento não é verificado quando avaliadas as sequências individualmente. Sugere-se a aplicação desta metodologia relevando as limitações apontadas, usando outras sequências de pulso diferentes e substituindo a WFS T2* por uma sequência de saturação de gordura por pulso de inversão.

Esta metodologia de análise, após validação por amostras de maior dimensão, poderá produzir

valores de referência para os limiares do stress muscular que permitam a distinção entre stress

comum de situações patológicas por sobrecarga excessiva de atividade física.

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ANATOMY AND GRAFTS – From Ancient Myths, to Modern Reality

ENXERTOS E ANATOMIA – Da Mitologia Antiga à Realidade Moderna

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FIG. 1 – CHIMERA, the modern icon of transplants – Chimera of Arezzo, Etruscan sculpture, c.380-360 BC – Archeological Museum, Florence
http://www.florence.museum.com.br/museu_arqueologico.php



ABSTRACT:

In medical terms, much is still to be learnt from ancient times' history. Such is certainly the case with the earliest historical signs of the concept of grafting and transplantations. Innumerable examples of zoanthropomorphic beasts and monsters, such as the Chimera, can be found throughout the world and in every culture, since the earliest times. In the Middle-Ages, we find several artistic representations of Cosmas and Damian, the patron saints of Medicine, transplanting the leg of a corpse in substitution of the amputated leg of a patient. Later, in the 16th Century, as we carefully analyzed the original text of Ambroise Paré's classification of prodigious living monsters and beasts, we detected what seems to be the first accurate scientific reference to the concept of regeneration of tissues after the first successful replantation and suture of the sectioned tendons of a human leg. In Leonardo da Vinci's biography, Vasari described that one of the first works of this extraordinary artist-scientist was the construction of an armored shield in which he built the representation of an imaginary frightening beast, that he constructed from different parts of the bodies of crawling insects and reptiles, collected in secrecy. In modern times, Mary Shelley's description of the monster Frankenstein is a clear allusion to the concept of grafting. These few selected historical examples seem to represent the constant scientific quest for the possibility of regeneration of tissues, inherent to grafting and transplantation that modern scientists are still in pursuit to perfect. **Keywords:** Anatomy, Grafts, History.

RESUMO:

O aprofundamento dos conhecimentos da História da Antiguidade muito pode ainda contribuir para o aperfeiçoamento técnico da Medicina Moderna. É esse, decerto, o caso das primeiras referências históricas aos conceitos de enxerto e transplantação. Um pouco por todo o mundo e em todas as culturas, é possível encontrar inúmeros exemplos de seres antropomórficos e monstros, como a Quimera. Na Idade Média, são várias as representações artísticas de Cosme e Damião, santos patronos da medicina, a realizar um transplante de perna de um cadáver em substituição da perna amputada de um doente. Mais tarde, no século XVI, a análise cuidada do texto original da classificação de “Prodígiosos monstros vivos e bestas” de Ambroise Paré, permite detectar aquela que pode ser considerada como a primeira referência científica coerente ao conceito de regeneração de tecidos, após um caso de sucesso de reimplantação e sutura dos tendões seccionados de uma perna humana. Vasari refere na biografia de Leonardo da Vinci, que uma das primeiras obras desse extraordinário artista-cientista foi a construção de um escudo com a representação de um assustador animal imaginário, construído com diferentes partes do corpo de insectos e répteis. Na era moderna, a descrição do monstro Frankenstein, por Mary Shelley, aparece como uma clara alusão ao conceito do sucesso de enxertos. Estes exemplos históricos parecem significar que, desde os primórdios dos tempos, sempre existiu uma permanente busca científica da possibilidade de regeneração de tecidos, através de enxertos e transplantes, que na medicina moderna continuam em vias de aperfeiçoamento.

Palavras-chave: Anatomia, Enxertos, História.

*“Je le soignait,
Dieu le guérit.”*
(Ambroise Paré, 1585)



INTRODUCTION

Much is still to be learnt from ancient times' history whenever, with due respect, we research what the History of Medicine and History of Art still have to offer in terms of the advances of medical technology. Such is certainly the case of the earliest mentions to the concept of grafting and transplantation.

Galen stated, in 200 AD, *On His Own Books*¹ [1], that Sciences and Humanities should not be studied independently. To-day, these statements remain an absolute truth, and modern physicians should thrive to perfection, not only through the

constant update of the innumerable technological advances, but also through the meticulous knowledge of the many historical facts and “hidden treasures”, offered by History in help of the resolution of the paradoxes of modern technology.

In this sense, we researched the important contributions of ancient mythology and History of Art, regarding the modern concept of grafting and transplantation.

In many circumstances, one cannot help feeling astonished at the precocious abundance of the earliest ideological signs of the concepts of grafts, implants and transplants, since the most remote stages of the history of Humanity.

As our researches progress, in the field of the history of transplants and grafts, it becomes clear that the concept of grafting between different

¹ “Most of those who want to study Medicine and Philosophy, and learn how to read texts correctly, will find, without previous thought, the books of those who taught both Sciences, the two greatest and most beautiful of Human Sciences.” Galen, “Sur mes Propres Livres”, in GALLIEN, Epitome en Quatre Parties. 1962, Union Latine d'éditions, Paris.

species was strongly prevalent in the minds of primitive cultures. The expression of fantasy in literature and arts demonstrates that, even though the concept of medical grafting is relatively recent (acquired in the 20th century), the idea of adding and substituting different parts of bodies between animal species is as old as the history of humanity [2].

THE CONCEPT OF GRAFTING IN ANCIENT MITHOLOGY

Primitive societies worshiped gods that were represented as animals. We can still find primitive cave paintings, illustrating bison, horses, bears, and deer, as central figures of hunting rituals, presumably as a form of recognition to these sacred

animals that were believed to represent a powerful source of vital energy to those that ate them [3]. The oldest surgery recorded in humans of the prehistoric archaeological records from the Bronze Age showed skulls being subjected to trephination: To relieve intracranial pressure, a circular disc of bone was removed from the calvaria and later replaced as an orthoptic autograft [4].

With the birth of the first civilizations, **in Egypt and Mesopotamia**, we discover artistic representations of zoo-anthropomorphic (half-animal, half-human) divinities of the early mythologies, such as the various zoomorphic divinities widely depicted in Egyptian art. (Fig. 2)[5] [6] [7]



Fig. 2 – Egyptian anthropomorphic divinities:

2a- Sculpture of *hyeracocephalus* Horus, Musée du Louvre, Paris;

2b- *Ramesses between Horus and Anubis*, fresco painting from the tomb of Ramesses I in the Valley of Kings (KV16), Egypt, c.1280BC; [5] [6]

Fig. 2c – The Lamassu from Khorsabad (c.720 BC), Limestone, Musée du Louvre, Paris. [6] These huge carvings of human-headed winged bulls, called “Lamassu”, or guardian spirits of the gate, believed to ward off evil spirits. guarded each of the entrances through which foreigners were obliged to pass on their way to the throne room. Similar Lamassu kept stony watch over the entrances of other palaces, such as that of Ashurmasipal II.

Nowadays, in museums, all over the World, we can find reliques of ancient Egyptian art, depicting the first pharaohs, and their divinities, such as the ibis-headed *Thot*, the god of scriptures; the cow-horned *Hathor*, goddess of female fertility and child-birth; the lamb-headed *Khnum* and *Harsaphs*; or the crocodile-headed *Sobek* [5].

Anubis, the divinity of the embalming, bore the head of a jackal. (Fig.2b)

Horus, the divinity of heavens and King of Pharaohs, was represented with the head of a hawk, or falcon. (Fig.2a, 2b) According to the Egyptian legend, Horus suffered the loss of an

eye in the combat against *Seth*, the horrific god of violence, hatred and envy. In a clear allusion to the possibility of regeneration of tissues after grafting, Horus recovered his eyesight after substituting the lost eye with the amulet of a serpent. From then on, Horus (and the Greek *Zeus*) would be represented with two different eyes, one in the form of the moon, the other of the sun.

In fact, in clinical terms, the first attempts to reconstruct body parts with local flaps date back

to c. 3000 BC, as recorded by the *Edwin Smith papyrus*². [8]

In **Mesopotamia**, the *LAMASSU* giant statues (Fig. 2c) [6], representing winged genie, built at the entrance of the palace of Khorsabad (c. 720 BC) can be visited at the *Musée du Louvre*; or from the Palace of Ashurmasirpal II, in the neo-assyrian period (883-859 BC), to be visited at the *Metropolitan Museum of Art*. These winged beings appear to be protective spirits and generally flanked images of the Assyrian king performing rituals. [9]

Several other anthropomorphic beasts are depicted in an interesting cylinder seal impression, from Ur, Iraq, (c.2700 BC), kept at the Oriental Institute of the University of Chicago. [10]

Greek mythology offers several anecdotes that immediately remind us how the Greek philosophers and medical practitioners had, in parallel with accuracy of anatomical knowledge, the

intrinsic concept of the possibility of grafting and transplanting [11-12-13]. Many of the heroes of the Pantheon were hybrids with zoo-anthropomorphic bodies. Greek tales introduce the strong fantasy of the concept of regeneration of the organic tissues of these hybrid divinities:

The Greek titan *Typhoeus* (*Typhon*), (identified with the Egyptian *Seth*), was half-dragon above the waist, and half-man, from the waist down. It had 100 heads, feathers covering his back, and a tail covered with snakes. Zeus attacked him with lightenings, to cut his nerves. Hermes and Pan came to rescue, and re-implanted his nerves.

Typhoeus and *Echidna*, half-woman, half-cobra, bore several monstrous offsprings, most of which also were hybrids:

- *Cerberus* - the three-headed dog, guards the entrance to Hades, indefinitely fought by *Heracles* (Hercules) in the latest of his renowned twelve labours;
- *Gorgon* - the immortal snake-haired humanoid was created in its mother's image. Its stare could turn a person to stone;

² [<http://archive.nlm.nih.gov/proj/ntp/flashsmithsmith.html>]

- *Hydra* - the nine-headed serpent, grew two new heads for every one that was cut off;
- And the *Sphinx* - the half-human, half-lion, forces those that it meets to answer its riddles, or die³. (Fig. 3a) [14]
- As many of the references to *Echidna*'s monstrous children immediately remind us of the concept of the regeneration of grafts, the monstrous fire-breathing *Chimaera*, considered by Hesiod ⁴ as one of *Echidna*'s offsprings, has become the modern icon of transplantation. (Fig. 1) [14]

Etruscan and early Roman art resumes some of the Greek mythological hybrid beasts, such as *Typhon* (Typhoeus), the *Centaur* (Fig. 3b) [14] or the *Chimera* (Fig. 1). From then on, mythological figures have been a recurrent theme in European and Western art and culture.

³ <http://www.greekmythology.com/>
<http://www.theoi.com/Bestiary.html>

⁴ Hesiod refers in the *Theogony* (pp. 319-329), after Homer's *Iliad*, that *Echidna* «...was the mother of the *Chimaera*, who breathed raging fire, a creature fearful, great, swift-footed and strong, who had three heads, one of a grim-eyed lion, in her hinder part, a dragon, and in her middle, a goat breathing forth a fearful blast of blazing fire...»

**Fig. 3-****3a-The Naxian sphinx**, Delphi, Greece, c. 540 BC. (Archaeological Museum of Delphi) [14]

This elegant representation of the Greek myth of the Sphinx reminds us of the many Egyptian sculptures of the sphinx that protected the entrance of the main Egyptian temples and tombs, such as the great Sphinx of Giza, protecting Khafre; or the Sphinx of Amenhotep III.

3b- Centaur being ridden by Cupid. 1st – 2nd century AD. (Musée du Louvre, Paris). [14]

This Roman masterpiece recurs the mythological theme of the *bas-relief* of the South mesotype of the Greek Parthenon (440-438BC), depicting the Battle of the Lapix and the Centaurs.

3c- Sculpture of AVALOKITESHVARA, Himalayan Monastery of Tabo, Spiti (15th C.) According to M. Singh (1968), the thousand arms of Avalokiteshvara whirl up in a wheel in the form of a shell. The thousand eyes, placed in the palm of each hand, reflect the infinite mysteries of the divinity. [15]

recorded in the Edwin Smith papyrus and Sanskrit

Searching around the globe for other mythological hybrids, one should not forget a short passage through the fabulous **Hindu mythology**.

The central figure of *Avalotishkevara* (Fig. 3c) [15], the Buddhist divinity that bears several heads and a great number of arms and eyes, immediately reminds us of Pearson's information, that the earliest attempts to reconstruct body parts with local flaps date back to approximately 3000 BC, as

texts from India[8]. Some of these flaps are still in

use today as the forehead flap for nasal reconstruction described in India by *Sushruta* 600 BC.⁵

The Hindu legend of *Ganesh* (the elephant-headed divinity of fortune and

⁵ <http://ia700305.us.archive.org/1/items/englishtranslati00susruoft/englishtranslati00susruoft.pdf>

prosperity) immediately comes to the mind of those who search for signs of the concepts of grafting and transplants in ancient mythologies⁶.

Bosch's extraordinary fantasies in painting. (Fig. 4) [19]

THE EVOLUTION OF THE CONCEPT OF GRAFTING IN THE MIDDLE AGES

It seems that during the Middle Ages, progress in flap surgery was slow, particularly in Europe, due to prevailing mysticism and religious proselytism. However, even in this period, Emperor Justinian II allegedly underwent nasal reconstruction after being overthrown. After this procedure, he was able to regain power [8][12][16][17].

In contrast, in the Arab world, progress in flap surgery was registered under the influence of Indian culture by Islam conquerors [18].

Interestingly, in Art, we find innumerable new examples of hybrids, such as the griffins in many capitals of Romanic cathedrals, or Hieronymus

⁶ According to the Hindu legend, *Ganesh* was the offspring of *Shiva* and *Parvati*. After a long absence from home, *Shiva* failed to recognize his grown-up son, and cut his head off. As *Parvati* reminded him of the adolescent's identity, *Shiva* was so afflicted by this that he ran to collect the first living creature's head that he found, and implanted the head of an elephant in substitution of the boy's own head.
<http://hinduism.about.com/od/lordganesha/a/ganesha.htm>



Fig. 4 – Hieronymus Bosch. *The Temptations of St. Anthony*. (c. 1510, Museu Nacional de Arte Antiga, Lisboa) (three details) [19]. This subject of the hybrid devils haunting St. Anthony, was resumed by several Renaissance artists, such as Schongauer (1490); Grünewald (c. 1515); Dürer (c. 1516); Bruegel (1526); and later on as a favourite subject of surrealist art, as with Max Ernst (1945), S. Dalí (1946); Diego Rivera (1947); or Don McBurney (1994)

Cosmas and Damian, the Christian patron saints of Medicine, were twin brothers, born in Arabia, who lived in Egaea, Cilicia (Turkey), and became eminent for their skill in the science of medicine. Under *Diocletian*, their prominence rendered them marked objects of persecution. Being apprehended by order of *Lysias*, governor of Cilicia, they underwent various torments that led them to die in martyrdom, about the year 283 AD.⁷

According to the legend, their most famous miraculous exploit was the grafting of a leg from a recently deceased Ethiopian to replace a

patient's ulcerated or cancerous leg, and the subject of many paintings and illuminations, such as the one by J. Huguet (1415-92), found in Santa Maria of Egara in *Terrassa*, Barcelona, Spain. Several other representations of this miraculous cure contributed to the fame of these two martyr saints, such as the works by Matteo di Puccino (1350-75); by Fra Angelico (c.1438) (Fig.5a); by F. del Rincón (c. 1450-1517); or by Mestre de los Balbases (1495)⁸(Fig. 5b) [20]

⁷ http://www.catholic.org/saints/saint.php?saint_id=471

⁸ <http://www.liveinternet.ru/users/4168247/post281222888/>

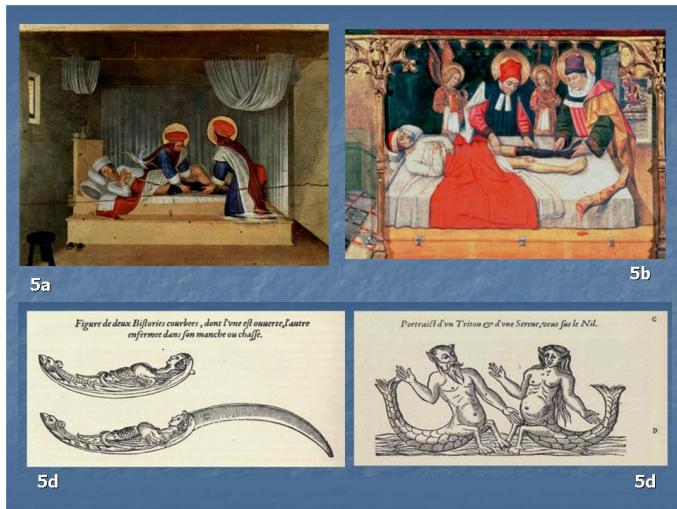


Fig. 5 - SS. Cosmas and Damian graft the leg of an Ethiopian man onto the stump of deacon Justinian;

5a- Fra Angelico , c. 1438 [http://www.delou-chiquito.com/2011_10_01_archive.html] ; **5b-** Jaume Huguet (1415-92). Santa Maria of Egara in Terrassa, Barcelona, Spain

Fig.5c – Ambroise Paré’s curved surgical scalpels. (Illustration on page CCCIII of the 8th Book of Paré’s 10 Books of Surgery) - Notice the carving on the handles of the scalpels, with zoomorphic feminine figurines. [23]

5d – Ambroise Paré – Illustration to the chapter of the Sea Monsters, (from page MLXVI, of the 25th Book, *Des Monstres et Des Prodiges* -The Complete Works of Ambroise Paré de la Vale au Maine, 1585) [23]

THE RENAISSANCE TIMES

In Leonardo da Vinci’s biography, Vasari (1585) described [21]⁹ that one of the first works of

this extraordinary artist-scientist was the construction of an armored shield in which he built the representation of an imaginary frightening beast, that he constructed from different parts of the bodies of crawling insects and reptiles, collected in secrecy. [21]

In 1506, Leonardo dedicated one of his studies to the battle of St. George against the dragon¹⁰.

⁹ VASARI (1585) described in Leonardo’s Biography that: «...It is said that Ser Piero da Vinci was at his country villa when he was sought by one of his peasants, who had with his own hands made a small round shield from the wood of a fig tree, and who wanted Ser Piero to have it painted in Florence. And so he had it taken to Florence and, without saying anything else to Leonardo about who’s in was, he asked him to paint something on it. Leonardo [...] after having it covered with gesso and prepared it in his own manner, he began to think about what he could paint on it that would terrify anyone that encountered it, and produce the same effect as the head of the Medusa. Thus, for this purpose, Leonardo carried into a room, crawling reptiles, green lizards, crickets, butterflies, locusts, bats, and other strange species of this kind and, by adapting various parts of this multitude, he created a most horrible and frightening monster with poisonous breath that set the air on fire. And he depicted the monster emerging from a dark and broken rock, spewing forth poison from its open mouth, fire from his eyes, and smoke from his nostrils, so strangely that it seemed a monstrous and dreadful thing

indeed. [...]» (transcription from Bondanella’s translation of Giorgio Vasari’s *The Lives of the Artists* (1998) , pp. 288-289. [22]

¹⁰ Leonardo da Vinci *Studies of St. George and the Dragon* (Pen and ink, c.1506, Windsor Castle, Royal Library)

In the **16th century**, we find the transition between the barber-surgeon and the scientific medical practice, in the figure of **Ambroise Paré**, considered as *the Father of Modern Surgery*, after the publication of his Medical Texts (Fig. 5c; 5d).

[23]

As we carefully analyzed the original text of Ambroise Paré's scientific description and classification of prodigious living monsters and beasts [24], we detected what seems to be the first accurate scientific reference to the concept of regeneration of tissues, after successful replantation and suture of the sectioned tendons of a human leg.

¹¹

Not by mere chance, the scientific mind of Ambroise Paré included - amidst his comments and attempts to classify the collection of hybrid

monsters and “*Other Strange Things*” (Fig.8b) - an original medical description of the possibility of replantation of a leg, with regeneration of tendons and nerves, after the careful surgical suture of the wound. In fact, when we consider the great amount of pages that Paré used in his Medical Book to describe the treatment of wounds and amputations, it seems quite clear that this reference to the possibility of suture and tendons regeneration, by the inventor of artificial limbs, in a book dedicated to the description of hybrids and «other strange things», carries the weight of the first accurate scientific reference to the possibility of success of transplantation and grafting, as a possible treatment to limb amputations.¹²

¹² Ambroise Paré [1585 –(1964)] *Des Monstres, des Prodiges et des Voyages*. [24]

Chap. XVIII – «DE PLUSIEURS AUTRES CHOSES ÉTRANGES: [...] Étienne Tessier, maître barbier chirurgien demeurant à Orléans, homme de bien, et expérimenté en son art, m'a récité que, depuis peu de temps avait pansé et médicamenté Charles Veriguel, sergent demeurant à Orléans, d'une plaie qu'il avait reçue au jarret, partie droite, avec incision totale des deux tendons qui fléchissent le jarret ; et pour l'habiller, lui fit fléchir la jambe, en sorte qu'il cousit les deux tendons bout à bout l'un de l'autre, et la situa et traita si bien que la plaie fut consolidée, sans demeurer boiteux ; chose digne d'être bien notée au jeune chirurgien, afin que lorsqu'il lui viendra entre ses mains telle chose, il en fasse de semblable. [...] Et, pour conclusion, je dirai avec Hippocrate (Père et auteur de la Médecine) qu'aux maladies il y a quelque chose de divin, dont l'homme ne saurait donner raison. »

¹¹ Ambroise Paré [1585 –(1964)] *Des Monstres, des Prodiges et des Voyages*. [24]

In the «Appendice au Livre des Monstres», Paré quotes Plinius: “DES MONSTRES MARINS: Il ne faut douter qu'ainsi qu'on voit plusieurs monstres d'animaux de diverse façon sur la terre, ainsi qu'il en soit en la mer d'étrange sorte, desquels les uns sont hommes depuis la ceinture en haut, nommés Tritons, les autres femmes, nommées Sirènes, qui sont couvertes d'écaillles – ainsi décrit Pline – sans toutefois que les raisons que nous avons alléguées par ci-devant, de la commixion et la mélange de semences, puisse servir à la naissance de tels monstres... » (Fig.9b)

Throughout the Renaissance, and till today, many artistic movements have been dedicated to the illustration of ancient mythologies, including paintings, drawings and sculptures of hybrids. The lists would be endless, and for this purpose, we will only quote Piero di Cosimo's masterpiece on *Perseus and Andromeda*, c. 1513¹³

PRIMITIVE CULTURES

Zoo-anthropomorphic creatures are quite abundant in indigenous art and crafts, all over the World, still co-existing with modern occidental civilizations.

In 2006, we had the pleasure of visiting the recently renewed collection of indigenous art of the *Musée du Quai Branly*, in Paris¹⁴. The Musée exhibits numerous examples of hybrid, half-human, half-animal figures and masks, as those found by the Korrigane expeditions to New Guinea in 1934-1939, or the exhibit of African statuettes and sculptures brought from Gabon, Nigeria or Benin in

the late 19th century. Those immediately remind us of the timeless ubiquity of the notions of metamorphosis that lye in the heart of mythical beliefs and their artistic expressions, in every human culture.

From the Oceania¹⁵, and the Pacific regions of the Melanesia, Micronesia, or Polynesia, we find the astonishing crafts of the *Vanuatu* people, from the Malakula Island, originally inhabited by cannibals, who built the *Ramparamp*, ancestral funerary effigies of high-graded men, from which the head skeleton was recovered at the time of death and prepared to build the funerary effigy. The human skull is overmodeled with vegetable matter and painted with natural pigments. The body consists of a vegetable fiber paste, decorated with bark belt, breast ornaments, shells, spider webs, pigs' teeth, and any other imaginable variety of materials, to reproduce the human expression. The rest of the effigy of the human figurine is built with all the variety of vegetable fibers, including dried

¹³

http://www.virtualuffizi.com/uffizi1/Uffizi_Pictures.asp?Contatore=123
<http://ferrebeekeeper.wordpress.com/2010/06/17/perseus-rescuing-andromeda-by-piero-di-cosimo/>

¹⁴ [http://www.quaibranly.fr/fr/collections/promenades-a-la-carte/rites-funeraires.html?tx_fepromenadealacarte3_pi1\[uid\]=851](http://www.quaibranly.fr/fr/collections/promenades-a-la-carte/rites-funeraires.html?tx_fepromenadealacarte3_pi1[uid]=851)

¹⁵ <http://wamena-gallery.com/en/tribal-art/oceania/overmodeled-ancestor-skull-vanuatu~60~530.html>
(also: <http://www.tribalartbrokers.net/praisetribal/?p=519> ;
<http://www.flickr.com/photos/51293088@N05/8170144584/> or
<http://www.learner.org/courses/globalart/work/191/index.html>)

banana leafs, and the resulting human sculpture with the height of the dead corpse is used as a funerary mannequin to stand at the entrance of the deceased home.

GRAFTING AND TRANSPLANTS IN MODERN ART

One should not finish this short passage through the World of Art, without mention to what modern art (including the *Seventh Art*) has to offer to the subject of Grafts and Transplants.

Following the major scientific advances of the modern surgical techniques, the **Surrealists**, from the beginning of the 20th C. offered us some of the most inventive and picturesque illustrations of modernized hybrids. René Magritte's *Collective Invention*, twists our mind, depicting the «reverse mermaid» (Fig. 6), as a good example of how the human mind seems to have intrinsic ideas on the figures of grafting and hybrid transplants. Also, Salvatore Dali's work seems «haunted» by hybrid distorted images (Fig.15) [26].

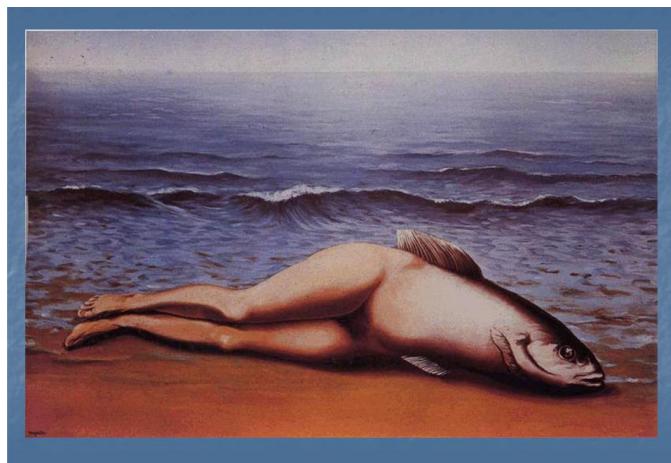


Fig. 6 - Magritte - *The Collective Invention*, 1934. <http://karlshuker.blogspot.pt/2011/03/rene-magritte-and-reverse-mermaid-very.html>

As if to help us add a note of conclusion to our nearly endless journey through the artistic illustrations of the concept of grafting and transplanting, the modern mind would immediately

«stump» on **Mary Shelley**'s writings on *Frankenstein, or the Modern Prometheus* [27], followed by several cinematographic sequels that render the intrinsic concept of drafting and

transplanting, as a closer and nearer reality, when compared to the ancient myths that we have been reporting here.

As we contemplate images from the original Boris Karloff's construction of the lumbering, fearsome monster Frankenstein, in the Universal's 1931 adaptation of Shelley's Novel¹⁶, we clearly realize that in terms of grafting and transplantation, ancient myths are soon to become a better modern reality in Medicine and Surgery.

CONCLUDING REMARKS – THE STATE OF THE ART

Even as we write, some artist in the world will be sculpting, painting or fantasizing on hybrid zoo-anthropomorphic figure, as the theme seems to haunt the human mind since the beginning of times, in parallel with the constant scientific quest for the possibility of regeneration of tissues, inherent to grafting and transplantation. The few selected examples collected from the history of Art around the world, that we present here, seem to reflect the desire to reach for eternal life, even if only through

the substitution of parts of the human body, with grafts and transplants that modern scientists are still in pursuit to perfect.

In modern dentistry, bovine bone xenografts are currently a well-accepted reality.^[28] A purified mineral matrix of bovine origin, mixed with the matrix of pig dental enamel is used as a substitute for bone tissue, produced by the chemical extraction of the organic matter of bovine bone, quite similar to the inner substance of human bones.

[29]

Nowadays, in plastic and reconstructive surgery, autologous grafts and flaps are being performed routinely all over the world for reconstructing missing parts of the body, after burns, trauma, congenital defects, tumor extirpation, infections, gender reassignment surgery, and even for aesthetic purposes. However, this was not always the case, obviously.

Although the first description of skin grafting probably dates back to ancient Indian scrolls, current skin grafts were based of Barionio's work on skin flaps in sheep entitled "*On grafting in Animals*" in 1804. Skin grafts, albeit apparently

¹⁶ <http://uk.ign.com/articles/2012/11/01/the-top-25-horror-movie-villains> ; <http://youtu.be/-voWJkaRWrU>

simpler to perform than skin flaps, were only put in routine clinical use after the description of partial skin grafting by Reverdin in 1869. [8] From then on numerous progresses and refinements have been made in graft surgery, encompassing not only skin, but also bone, tendon, fat, nerve, cartilage, and mucosa alone or in multiple combinations. [18]

In the **20th century**, especially after the World Wars, surgeons were faced with a massive number of injuries, many of which were rather complex. This spurred the development of numerous random and axial flaps.[8] Up to the present day, many of these flaps have been perfected, as a result of a better anatomical understanding. [30-35]

In parallel, Alexis Carrel's work on vascular anastomoses technique led to the development of free transfer of tissues, which granted him the first Nobel Prize earned by a surgeon in 1912. This seminal work allowed the development of replantation procedures. In fact, Alexis Carrel performed the first extremity replantation in a

complete amputated canine hind limb in 1906 [36-37].

This work paved the way to limb replantation [37-38]. The first arm replantation was performed in 1962 by Malt, the first hand replantation by Chen in 1963 and the first digital replantation in 1968 in Japan [39-41]. Since these early days this field grew exponentially, reaching an astounding degree of complexity. Presently, limb replantation is being performed routinely all over the world [39][42][43]. Moreover, replantations of increasingly mangled and distal amputations are being performed every day. [37]

Free flaps, in which a body part is transferred based on its vascular pedicle to a different place where there is a “defect”, were first performed by Cobett in 1968. This surgeon transferred toes as free flaps to reconstruct missing digits in mangled or congenital malformed hands, rivaling ancient mythological musings [41][44-47]. In 1972, Taylor introduced a different concept of reconstructing vital parts of the body with redundant distant tissues, using the free groin flap.

From then on, almost every angiosome of the body has been transferred alone or in combination to reconstruct a previously unimaginable array of defects [41][44-47]. Some of these combinations are so strange as using the latissimus dorsi muscle flap to reconstruct the entire dorsal surface of the foot and most of the midfoot bones [48]. Oddly, many of these reconstructions have been crowned with pleasing results [48-49].

Recently, there has been a trend to associate the free transfer of nonvital organs from brain dead donors as free flaps with increasingly better immunosuppression protocols. [8] This has allowed the successful transference of the face, hand, forearm, arm, lower and upper leg, and even larynx and trachea from cadavers [8] [50-60]. In fact, skin xenografts and allografts, as well as synthetic skin

substitutes have been used regularly for the past decades in burn patients [61-66].

Finally, **in the last three decades**, different vascular territories have been associated with different distant tissues in order to create true living bioreactor to tailor make pre-fabricated flaps to reconstruct complex defects, namely those involving the airway, gastrointestinal, and airway tracts or those involving extensive injuries to the face or limbs [8] [51].

In this exciting new era in which almost everything seems possible, one cannot help noticing how much of what is now current reconstructive practice was inspired in the ancient myths and dreams of our predecessors.

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