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Sumário / Summary

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Referência da capa: Vénula pós-capilar (diâmetro aproximado: 30 mm) de rede microvascular em mesentério de rato (*Rattus norvegicus*), observada por microscopia intravital de transiluminação. No interior do vaso sanguíneo visualizam-se leucócitos a interagir com a parede vascular. Imagem obtida por Henrique Sobral do Rosário (Instituto de Biopatologia Química – Prof.a Doutora Carlota Saldanha, Faculdade de Medicina de Lisboa; Unidade de Biopatologia Vascular, Instituto de Medicina Molecular)

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HEMORREOLOGIA REVIGORADA/HEMORHEOLOGY RENEWAL

Realizou-se este mês de Junho a "16th Conference of the European Society of Clinical Hemorheology and Microcirculation", em Munique, cuja notícia e contributo da nossa sociedade podem ser consultados neste boletim.

A ciência hemorreológica evoluiu substancialmente como resultado da influência do crescimento dos grupos nos diferentes países e da associação com áreas afins que ajudam a esclarecer algumas dúvidas mas, sobretudo, porque levantam novas questões.

Este congresso foi um marco para a revisão e apresentação de novos trabalhos nos domínios da Hemorreologia e da Hemostase no que respeita (i) à cirurgia de bypass da artéria coronária, e circulação extracorporal, (ii) à terapias por transplante, por fibrinogenioferese, por substituição hormonal, contracepção, anticoagulação, antifibrinolitica e antiplaquetária, (iii) aos estudos clínicos transversais e longitudinais de tromboembolismo venoso, de acidentes agudos e eventos da circulação coronária e (iv) aos ensaios de diagnóstico com meios de contraste a nível da microcirculação.

A associação entre a hemorreologia e a reactividade vascular foi revista na diabetes, na hipertensão arterial, no exercício físico, na obesidade e no envelhecimento.

Os resultados controversos obtidos no passado e no presente foram salientados como pontos de partida de novas hipóteses para futuras abordagens experimentais a nível molecular. As vias de transducção de sinal de mecanismos moleculares foram debatidas e, consequentemente, serão objectivos de futuros trabalhos e aparecerão nas próximas publicações.

Intaglieta (San Diego, USA) questionou a utilização das transfusões sanguíneas versus a eficiência dos fluidos expansores plasmáticos, salientando os benefícios dos que apresentam superior viscosidade. Também nesta linha de pensamento Gori, do grupo de Sandro Forconi, apresentou os resultados de trabalhos que apontam para a associação negativa entre a viscosidade sanguínea e a pressão arterial. Salientou Gori que, à primeira vista, parece haver uma contradição com os benefícios das terapias hemorreológicas de diminuir a viscosidade sanguínea nas doenças cardiovasculares.

No entanto, parafraseando-o *"that terms such as hemorheological deterioration and endotheliun dysfunction have been superficially used in the past, forgetting that what appears to be negative might simply be the result of a physiological, protective response"*. Nesta sequência foram sugeridas várias ideias explicativas, uma das quais relacionada com os tipos enzimáticos da sintase do monóxido de carbono presentes na célula endotelial na presença e ausência de resposta inflamatória. Foram também

apresentados neste congresso alguns resultados de trabalhos clínicos com foco na Inflamação e Hemorreologia os quais têm sido uma constante da nossa investigação desde a década de 80 do século passado.

Outra componente apresentada ligada à Hemorreologia foi a dos estudos desenvolvidos para melhorar a microcirculação. Os resultados obtidos, por Rossi (Universidade de Pisa, Itália) em doentes obesos submetidos a cirurgia gástrica são promissores pelas repercussões positivas na redução dos factores de risco cardiovascular. Novos sistemas de compressão desenvolvidos pelo grupo de Arnold e colaboradores (Universidade de Greifswald na Alemanha) foram aplicados com sucesso a doentes com doença oclusiva arterial periférica, que melhoraram os parâmetros indicadores da funcionalidade da microcirculação. Também o grupo de Géis (Hospital Universitário de Regensburg na Alemanha iniciou um "clinical trial" com uma nova técnica de contraste (contrast enhanced ultrasound, CEUS) de monitorização fácil da avaliação de perfusão muscular, cujos resultados preliminares permitem detectar deficiências microcirculatórias em doentes sob intervenção cirúrgica ou excluídos dela ou, ainda, em verificação do sucesso pós-cirúrgico.

A Hemorreologia está revigorada pela necessidade de prevenir e aliviar as doenças vasculares, tão dependentes das interligações entre sangue e vasos e dos sistemas de controlo metabólico, neuronal e hormonal.

A SPHM, como sempre, está receptiva a novos estudos e a novos sócios. Consulte o *site*, escreva para o blogue, estabeleça contacto, pois a SPHM está também ligada às sociedades europeias.

Carlota Saldanha
Presidente da SPHM

CARDIO-RENAL ANTI-APOPTOTIC AND PRO-PROLIFERATIVE EFFECT OF RECOMBINANT HUMAN ERYTHROPOIETIN IN A MODERATE STAGE OF CHRONIC RENAL FAILURE IN THE RAT

Teixeira AM[1,2], *Garrido P*[1], *Rodrigues-Santos P*[2], *Costa E*[3,4], *Parada B*[1], *Sereno J*[1], *Mascarenhas-Melo F*[1], *Alves R*[5], *Belo L*[4,6], *Santos-Silva A*[4,6], *Teixeira F*[1], *Reis F*[1]

ABSTRACT

We hypothesize that recombinant human erythropoietin (rhEPO) therapy under circumstances of moderate chronic renal failure (CRF), with lower kidney and heart lesion/impairment, may have a protective effect beyond the correction of anemia, whose mechanism deserve better elucidation by clarifying the impact on cardio-renal gene expression profile on markers of apoptosis, inflammation, proliferation, angiogenesis and lesion/stress. Four groups of rats were studied over a period of 15 weeks (n=7 each): control – without surgery and without drug treatment; rhEPO – treated with 50 IU/kg/week of rhEPO-beta; CRF – submitted to partial nephrectomy (3/4); CRF+rhEPO – CRF with rhEPO treatment after the 3rd week of surgery. The kidney and the heart were collected in order to evaluate the gene expression, by real-time qPCR, of markers of apoptotic machinery (Bax, Bcl2, Fas, Faslg and caspases 3 and 9), inflammation/immunology (TNF-α, NF-Kβ and IL-2), proliferation/angiogenesis (TGF-β, VEGF and PCNA) and lesion/stress (cytochrome c and NOS2 and NOS3). The main finding obtained were: a) – CRF rats has demonstrate overexpression of EPO-R in the heart ($P>0.005$) and a trend to higher values in the kidney, without changes on EPO expression, together with overexpression of Bax/Bcl2 ratio, PCNA and IL-2 in both tissues ($P<0.005$ vs control); b) – rhEPO therapy on the remnant kidney and on the heart of

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the rats with CRF induced by partial 3/4 nephrectomy promoted non-hematopoietic protection, demonstrated by the apoptosis prevention in both tissues, viewed by the Bax/Bcl2 balance (P<0.05 vs CRF), by the promotion of proliferation, due to PCNA increment, particularly in the heart (P<0.01 vs CRF), and by the immunomodulatory action, expressed by the prevention of IL-2 increment in the remnant kidney (P>0.01 vs CRF). These effects were accompanied by a trend to higher values of EPO gene expression in the heart and of EPO-R in the remnant kidney and in the cardiac tissue. In conclusion, in this model of moderate CRF, rhEPO treatment showed important cardio-renal non-hematopoietic effects, expressed mainly by the anti-apoptotic and the pro-proliferative action. This data suggest that early rhEPO in moderate stages of CRF, before critical lesion of the tissues, might be a therapeutic measure with further benefits.

Key-words: moderate chronic renal failure, cardio-renal protection, gene expression, poptosis, inflammation, proliferation/angiogenesis, lesion/stress, animal model

INTRODUCTION

The appearance and development of chronic kidney disease (CKD) is often associated to a state of low levels of erythropoietin (EPO) production, due to loss of peritubular cells of the kidney, responsible for its formation, with consequent reduction in erythropoiesis, resulting in anemia[1]. The reduction of tissue oxygenation then causes an increase of cardiac function, and subsequent left ventricular hypertrophy, in order to reply to the oxygen demands by the peripheral tissues, which accounts for subsequent development of heart failure, a triad of dysfunctions is known as the cardio-renal anemia syndrome[1-3]. The relationship between the anemia-secondary to kidney disease and the heart failure is already well described and accounts for the high morbidity and mortality rates found in CKD patients[4,5].

The use of recombinant human erythropoietin (rhEPO) has emerged in the early 90s as the most appropriate method to control anemia in patients with CRF, and was responsible for a better and longer life in those patients, both from amelioration of anemia and beneficial cardiovascular impact[4-6]. Therefore, EPO has been recognized as a key player in a broad variety of processes in cardiovascular pathophysiology, including apoptosis, cell proliferation, ischaemia and the nitric oxide pathway, which give particular relevant to their use in non-haematological conditions[7]. However, for a significant percentage of patients, rhEPO losses efficacy, becoming resistant, recommending dose increment with further deterioration of heart function, most probably due to the expected hyperviscosity and thromboembolisms[7-10]. Therefore, although enhanced EPO synthesis is viewed as an appropriate compensatory mechanism in the cardio-renal syndrome, excessive EPO synthesis in the advanced stages of both the chronic renal failure and congestive heart failure appears to be predictive of higher mortality[10]. Thus, early rhEPO use, in stages of yet moderate renal failure and low car-

diac deterioration, might have additional benefits. However, this impact remains poorly investigated.

Considering that the use of human tissues is obviously limited by ethical reasons, our group has previously extensively characterized a model of moderate chronic renal failure, which is a good tool to assess the pathological evolution of the triad, as well as to analyze the beneficial impact of early rhEPO use[11]. The model, induced by partial (3/4) nephrectomy, was consistent with a moderate but maintained degree of chronic renal failure (CRF), together with transitory anemia and iron metabolism disturbances. The remnant kidney presented a reasonable degree of functionality, mainly due to hypertrophic compensation, and there was several important cardiovascular modifications, including hypertension, tachycardia, dyslipidemia, erythropoietic disturbances, sympathetic activation, proliferation, angiogenesis and oxidative stress, which are features seen in CKD patients[11,12].

Using our model, we found that rhEPO treatment in the moderate CRF promotes erythrocytosis and prevented tachycardia, catecholamines increment and dyslipidemia, with a rise of serum TGF-β1. Furthermore, the decreased kidney gene expression of EPO and the overexpression of Caspase 9 were prevented, demonstrating a renoprotective action on the remnant kidney[12,13]. Thus, rhEPO therapy has promoted a protective effect on the cardio-renal axis, which might be attributed to its protective actions, namely of pro-proliferative and anti-apoptotic nature, as was previously suggested in other conditions and pathologies[3-6], but that deserves a more extensive evaluation, particularly in moderate stages of CKD, before critical impairment of the kidney and heart.

EPO gene activation, mainly by hypoxic states, is linked with several important pathways, with unequivocal impact on renal and cardiac tissues function and structure. The 3' end of the EPO gene contains an element of response to situations of hypoxia (HRE), which interacts with multiple transcription factors, including hypoxia-induced factor (HIF), a regulator of the genes that are involved in adaptation to alterations in oxygen levels. The HIF will act on several genes, including vascular endothelial growing factor (VEGF) and the glucose transporter GLUT-1[14]. Although the regulation of erythropoiesis occurs mainly in the peritubular cells of the kidneys, 10% of EPO is expressed in extrarenal tissues such as in liver, brain, spleen and lungs[15-17]. Binding of EPO to its receptor (EPO-R) causes a change in intracellular calcium levels, including the formation of IP3 that is able to inhibit the occurrence of apoptosis due to activation of its effector, Akt, an apoptotic blocker involved in proliferation and cell survival[16]. The interaction between the EPO and its receptor also leads to activation of ras/MAPK signaling pathways, by activating nuclear factor kB (NF-kB), with consequent strengthening of cell proliferation[15-17].

Our previous findings concerning a protective role of rhEPO, together with the unequivocal impact of EPO on pivotal signalling pathways for apoptosis, proliferation, angioge-

nesis and inflammation, was the basis for the present study, which was designed to assess the effects of rhEPO on gene expression profile on the kidney and heart in a moderate stage of moderate CRF. We hypothesize that rhEPO under that circumstances of lower tissue lesion/impairment may have a protective effect beyond the correction of anemia, whose mechanism deserve better elucidation by clarifying the impact on gene expression profile of several markers of apoptosis, inflammation, proliferation, angiogenesis and lesion/stress.

MATERIALS AND METHODS

Animals and diets

Male Wistar rats (Charles River Lab. Inc., Barcelona, Spain), weighing ± 275g, were maintained in an air conditioned room, subjected to 12-h dark/light cycles and given standard rat chow (IPM-R20, Letica, Barcelona, Spain) and free access to tap water. Animal experiments were conducted according to the European Communities Council Directives on Animal Care. The rats were divided into 4 groups (7 rats each), during a 15-week protocol: control – without drugs and surgery; rhEPO (beta) – 50 IU/Kg/week s.c. Recormon® (Roche Pharmaceuticals), without surgery; CRF – induced by a two-stage (3/4) nephrectomy: firstly, about half of the left kidney was removed and, one week later, the entire right kidney was removed; CRF+rhEPO – treated with rhEPO after the 3rd week of surgery. All the animals have completed the protocol.

Kidney and heart collection and preparation

The rats were sacrificed by cervical dislocation, after intraperitoneal anesthesia with a 2 mg/kg BW of a 2:1 (v:v) 50 mg/mL ketamine (Ketalar®, Parke-Davis, Lab. Pfeizer Lda, Seixal, Portugal) solution in 2.5% chlorpromazine (Largactil®, Rhône-Poulenc Rorer, Lab. Vitória, Amadora, Portugal). The heart and the kidneys were immediately removed, placed in ice-cold Krebs' buffer and carefully cleaned of adherent fat and connective tissue, freezing therefore in RNAlater tubes at –80ºC.

Kidney and heart gene expression analysis

Total RNA isolation: Kidneys and heart were isolated in autopsy and stored in RNA later™ solution (Ambion, Austin, USA). Samples were removed from preservation solution and 1200 µl of RLT Lysis Buffer were added to proceed with disruption and homogenization for 2 minutes at 30Hz using TissueLyser (Qiagen, Hilden, Germany). Tissue lysate were processed according to the protocol from RNeasy® Mini Kit (Qiagen, Hilden, Germany). Total RNA was eluted in 50 µl of RNase-free water (without optional treatment with DNAse). In order to quantify the amount of total RNA extracted and verify RNA integrity (RIN, RNA Integrity Number), samples were analyzed using 6000 Nano Chip® kit, in Agilent 2100 bioanalyzer (Agilent Technologies, Walbronn, Germany) and 2100 expert software, following manufacturer instructions. The yield

from isolation was from 0.5 to 3 µg; RIN values were 6.0-9.0 and purity (A_{260}/A_{280}) was 1.8-2.0.

Reverse Transcription: RNA was reverse transcribed with Super-Script™ III First-Strand Synthesis System for RT-PCR (Invitrogen, California, USA). One microgram of total RNA was mixed with a 2X First--Strand Reaction Mix and a Super-Script™ III Enzyme Mix (Oligo(dT) plus Random hexamers). Reactions were carried out in a thermocycler Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, USA), 10 min at 25ºC, 50 min at 50ºC and 5 min at 85ºC . Reaction products were then digested with 1 µl RNase H for 20 min at 37ºC and, finally, cDNA eluted to a final volume of 100 µl and stored at –20ºC.

Relative quantification of gene expression: Performed using 7900 HT Sequence Detection System (Applied Biosystems, Foster City, USA). A normalization step preceded the gene expression quantification, using geNorm Housekeeping Gene Selection kit for *Rattus norvegicus* (Primer Design, Southampton, UK) and geNorm software (Ghent University Hospital, Center for Medical Genetics, Ghent, Belgium) to select optimal housekeeping genes to this study[18]. Real-time PCR reactions used specific QuantiTect Primer Assays (Qiagen, Hilden, Germany) with optimized primers for TGF-β1 (QT00190953) and PCNA (QT00178647), as a proliferative markers; vascular endothelial growing factor (QT00198954) as a angiogenesis marker; a synthase 2 (inducible) and 3 (constitutive, endothelial) from nitric oxide, NOS2 (QT00186340) and NOS3 (QT01570618) as indicators of endothelial and constitutive enzyme activity; Cytochrome C (QT00366205) as a vascular damage factor; IL-2 (QT00185360), NF-kB (QT01573334) and TNF-α (QT00178717) as inflammatory markers; and at least apoptotic indicators such as caspase 9 (QT00188734), caspase 3 (QT01794429), Bax (QT01081752), Bcl2 (QT00184863), Fas (QT00196595) and Fas ligand (QT00178171). Endogenous controls were also used: GAPDH (QT00199633), ACTB (QT00193473), TOP1 (QT01820861) and RPL13 (QT00178675) together with QuantiTect SYBR Green PCR Kit Gene expression (Qiagen, Hilden, Germany) according to manufacturer's instructions. RT-qPCR reactions were carried out with: 100ng cDNA sample, primers (50-200 nM) and 1X QuantiTect SYBR Green PCR Master Mix. Non template control reactions were performed for each gene, in order to assure no unspecific amplification. Reactions were performed with the following thermal profile: 10 min. at 95ºC plus 40 cycles of 15 seconds at 95ºC and 1 min. at 60ºC. Real-time PCR results were analyzed with SDS 2.1 software (Applied Biosystems, Foster City, USA) and quantification used the $2^{-\Delta\Delta Ct}$ method[19].

Statistical analysis

For statistical analysis, we used the GraphPad Prism, Version 5.0. Results are presented as means ± standard error of means (SEM). Comparisons between groups and between different times of evaluation were performed using two-way ANOVA and the Post´hoc Bonferroni test. Significance was accepted at p less than 0.05.

RESULTS

Effect of rhEPO on kidney and heart EPO and EPO-R mRNA gene expression

We found that rhEPO treatment, per se (rhEPO group), promoted a trend to overexpression of EPO gene in the kidney (Fig. 1A1), with a statistically significant (P<0.05) reduction of EPO-R gene expression (Fig. 1A2). In the cardiac tissue, rhEPO was able to significantly (P<0.05) increase EPO gene expression (Fig. 1B1), together with a trend to higher values of EPO-R (Fig. 1B2), when compared with the control animals (Fig. 1). In the CRF animals, no significant changes were encountered in EPO gene expression in both the kidney and heart tissues (Fig. 1A1 and 1B1, respectively), but a trend to higher values of EPO-R was found in the kidney and statistically significant elevated in the heart (Fig. 1A2 and 1B2), when compared with the control rats. rhEPO treatment in the CRF animals (CRF+rhEPO group) showed a trend to higher values of EPO gene expression in the heart (Fig. 1B1), without further changes when compared with the CRF rats without rhEPO therapy (Fig. 1).

Effect of rhEPO on kidney and heart mRNA gene expression of apoptotic markers

The following proteins were evaluated as markers of the apoptotic machinery: Bax, Bcl2, Fas, FasLg and caspases 3 and 9. Concerning the rhEPO treatment, we found in the kidney tissue a significant (P<0.001) increment in Bax gene expression and a trend to higher expression of

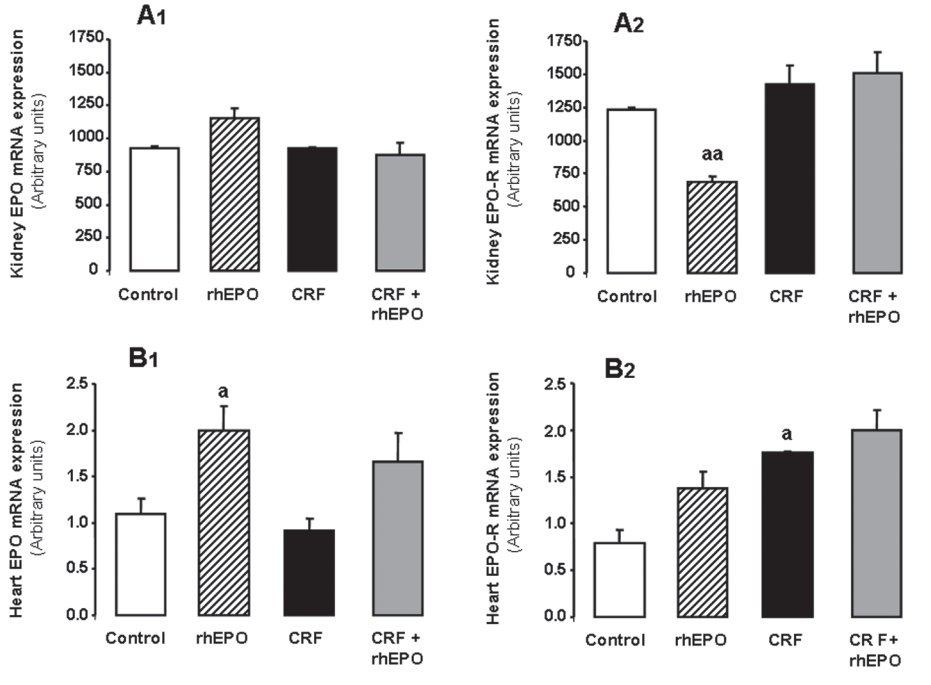

Figure 1 – Kidney (A) and heart (B) mRNA erythropoietin (1) and erythropoietin receptor (2) gene expression for the groups under study, at the final time. Results are means ± SEM (7 rats/group): $^{a}p<0.05$ and $^{aa}p<0.01$ *vs* the control group.

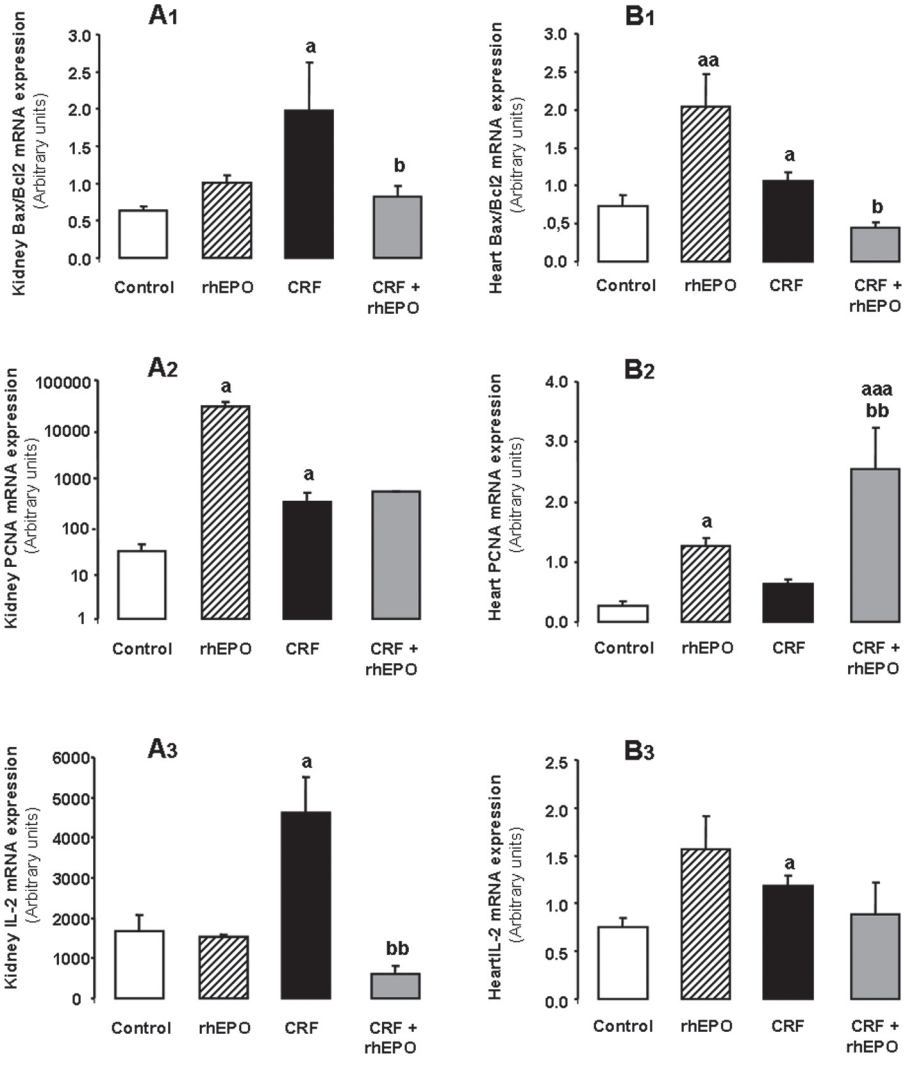

Figure 2 – Kidney (A) and heart (B) mRNA Bax/Bcl2 (1), PCNA (2) and IL-2 (3) gene expression for the groups under study, at the final time. Results are means ± SEM (7 rats/group): $^{a}p<0.05$, $^{aa}p<0.01$ and $^{aaa}p<0.001$ *vs* the control group; $^{b}p<0.05$ and $^{bb}p<0.01$ *vs* the CRF group.

Bcl2; Bax/Bcl2 ratio showed no significant changes when compared with the control (Fig. 2A1). No further changes were encountered in the other markers, including Fas, FasLg and caspases 3 and 9, despite a trend to higher expression of Fas, caspase 3 and 9 and lower of FasLg (Table I). In the heart tissue, however, rhEPO was able to promote a statistically significant increase (P<0.01) in Bax/Bcl2 ratio (Fig. 2B1), as a result of a trend to higher values of the pro-apoptotic protein Bax and lower of the anti-apoptotic Bcl2 (Table II). The other markers of apoptosis in the heart tissue were also unchanged, with again a trend to higher values of caspase 3 and 9 gene expression (Table II).

In the CRF animals, and concerning the kidney tissue, a statistically significant increment (P<0.05) in Bax/Bcl2 ratio (Fig. 2A1) was found, together with no further changes on the other proteins, Fas, FasLg and

Table 1 – Effects of rhEPO treatment on kidney mRNA gene expression of markers of apoptosis, proliferation, angiogenesis, inflammation and lesion/oxidative stress in a rat model of moderate CRF, at the final time (15 weeks)

Renal Parameters	Control (No surgery)		CRF (¾ Nephrectomy)	
Arbitrary units (AU)	**Vehicle**	**rhEPO**	**Vehicle**	**rhEPO**
Apoptosis				
Bax	567.45 ± 85.42	1,481.67 ± 104.93^{aaa}	679.40 ± 64.43	809.88 ± 119.40
Bcl2	897.15 ± 55.45	1.446,70 ± 176.75	400.05 ± 88.59	1,053.43 ± 194.95
Bax/Bcl2	0.63 ± 0.06	1.01 ± 0.11	1.98 ± 0.65^{a}	0.82 ± 0.15
Fas	1,007.55 ± 507,75	1,822.20 ± 171.83	1,626.45 ± 416.01	656.78 ± 329.77
Faslg	2,426.51 ± 707.54^{a}	938.30 ± 148.56	993.45 ± 351.92	1,133.08 ± 288.64
Caspase 3	956.35 ± 213.99	1,194.70 ± 145.64	656.65 ± 273.87	867.23 ± 69.87
Caspase 9	952.25 ± 84.15	1,582.51 ± 171.11	1,131.20 ± 271.82	739.08 ± 75.19
Proliferation and angiogenesis				
TGF-1β	1,130.10 ±102.13	1,335.02 ± 85.42a	790.60 ± 198.84	709.05 ± 86.21
Citocrom c	591.45 ±180.34	2,330.71 ± 177.98^{aaa}	550.95 ± 19.08	473.40 ± 100.32
VEGF	1,025.25 ± 231.95	991.35 ± 135.56	1,108.55 ± 294.71	934.43 ± 155.11
Inflammation/Stress				
TNF-α	1,273.01 ± 19.05	1,247.44 ± 85.94	1,152.05 ± 105.63	656.85 ±166.88^{a}
NF-Kβ	1,066.55 ± 272.19	1,442.20 ± 187.02	882.65 ± 107.59	751.83 ± 117.24
NOS2	696.75 ± 265.73	2,821.01 ± 384.21^{aa}	566.80 ± 99.77	371.18 ± 88.25
NOS3	2,210.50 ± 417.71	456.89 ± 76.88^{aaa}	3,159.01 ± 387.98	1,804.25 ± 183.21^{bb}

Results are means ± SEM (7 rats/group): ^{a} $p<0.05$, ^{aa} $p<0.01$ and ^{aaa} $p<0.001$ *vs* the control group; ^{bb} $p<0.01$ *vs* the CRF group.

caspases 3 and 9 (Table I). In the heart, the same pro-apoptotic profile concerning Bax/Bcl2 was found, with a significant increase (P<0.05) versus the control group (Fig. 2B1), without any other significant effect on Fas, FasLg and caspases mRNA gene expression (Table II).

Concerning the rats under rhEPO treatment with CRF (CRF+rhEPO group), in the kidney tissue there was a prevention of the pro-apoptotic effect promoted by the renal failure, with a statistically significant (P<0.05) reduction of Bax/Bcl2 ratio when compared with the animals without rhEPO therapy (Fig. 2A1). Once again, no further changes were found for the other parameters (Table I). In the heart tissue, a similar anti-apoptotic protection was encountered in Bax/Bcl2 ratio (P<0.05) (Fig. 2B1), due to a significantly increment (P<0.01) on Bcl2 gene expression (Table II).

Effect of rhEPO on kidney and heart mRNA gene expression of proliferative and angiogenic markers

To assess the influence of rhEPO treatment on kidney and heart proliferation and angiogenesis, we evaluated the following proteins: PCNA, TGF-β1, citocrom c and VEGF. Concerning the rhEPO treatment, per se (rhEPO group), we found a remarkable increment in PCNA mRNA

Table 2 – Effects of rhEPO treatment on heart mRNA gene expression of markers of apoptosis, proliferation, angiogenesis, inflammation and lesion/oxidative stress in a rat model of moderate CRF, at the final time (15 weeks)

Cardiac Parameters	Control (No surgery)		CRF (¾ Nephrectomy)	
Arbitrary units (AU)	**Vehicle**	**rhEPO**	**Vehicle**	**rhEPO**
Apoptosis				
Bax	0.89 ± 0.10	1.07 ± 0.08	1.11 ± 0.00	1.26 ± 0.17
Bcl2	1.47 ± 0.25	0.63 ± 0.16	1.09 ± 0.12	2.95 ± 0.39[aa, bb]
Bax/Bcl2	0.73 ± 0.15	2.04 ± 0.43[aa]	1.06 ± 0.12	0.45 ± 0.07
Fas	1.55 ± 0.39	0.96 ± 0.62	2.48 ± 0.11	3.91 ± 0.51
Faslg	0.53 ± 0.11	0.57 ± 0.18	0.73 ± 0.02	1.13 ± 0.42
Caspase 3	0.85 ± 0.07	1.12 ± 0.12	1.23 ± 0.15	1.20 ± 0.08
Caspase 9	1.28 ± 0.26	1.14 ± 0.21	0.66 ± 0.24	0.54 ± 0.04
Proliferation and angiogenesis				
TGF-1β	1.28 ± 0.23	1.43 ± 0.37	0.98 ± 0.31	0.60 ± 0.06
Cytochrome c	1.54 ± 0.29	0.82 ± 0.16	1.24 ± 0.22	0.70 ± 0.05
VEGF	1.15 ± 0.16	1.84 ± 0.67	2.08 ± 0.39	2.39 ± 0.10
Inflammation/Stress				
TNF-α	1.23 ± 0.19	1.46 ± 0.15	1.05 ± 0.31	1.25 ± 0.18
NF-Kβ	0.61 ± 0.09	1.17 ± 0.26	1.16 ± 0.01	1.29 ± 0.15
NOS2	0.62 ± 0.10	1.01 ± 0.19	0.51 ± 0.17	0.83 ± 0.03
NOS3	0.67 ± 0.09	1.12 ± 0.19	1.10 ± 0.19	1.45 ± 0.17

Results are means ± SEM (7 rats/group): [aa] $p<0.01$ *vs* the control group; [bb] $p<0.05$ *vs* the CRF group.

gene expression in both the kidney and the heart ($P<0.05$) (Figs. 2A2 and 2B2, respectively). We should mention that the values in the kidney changed from the control group of 25.91 ± 10.18 AU to 27,933.45 ± 7,251.23 in the rhEPO animals, whcih is an unequivocal increment. rhEPO treatment also promoted a trend to higher values of TGF--β1 gene expression in the kidney, together with statistically significant increment in citocrome c gene expression ($P<0.001$), without unchanges values of VEGF (Table I). In the heart, rhEPO treatment promoted no changes on TGF-β1, citocrome c and VEGF gene expression, despite a trend to higher values of TGF-β1 and lower of citocrome (Table II).

In the CRF animals, and concerning the kidney tissue, we found a notoriously higher value of PCNA (287.22 ±154.27 AU; $P>0.05$) when compared with the control animals (25.91 ± 10.18 AU) (Fig. 2A2). Similar patern was found for the cardiac tissue, with a tend to higher values (0.63 ±0.10 AU) versus the control rats (0.27 ± 0.07 AU) (Fig. 2B2). Concerning the other markers, no changes were encountered in the kidney and in the heart for TGF-β1, citocrome c and VEGF gene expression levels (Table I and II, respectivelly).

In the rats under rhEPO treatment with CRF (CRF+rhEPO group), PCNA gene expression was identical to that found in the kidney of CRF rats

and was even higher (P<0.01) in the heart (Fig. 2A2 and 2B2, respectivelly). No other significant chnages were encountered concerning the other proteins, both in the kidney and in the heart (Table I and II, respectivelly).

Effect of rhEPO on kidney and heart mRNA gene expression of markers of inflammation and lesion/stress

We also assess the influence of rhEPO treatment on kidney and heart IL-2, TNF-α, NF-KB and NOS2 and NOS3 gene expression. Concerning the rhEPO treatment, per se (rhEPO group), we found no significant changes for all the parameters analyzed in the heart tissue, despite a trend to higher expression in all of them (Fig. 2B3 and Table II). Concerning the kidney, rhEPO also promoted no changes on IL-2, TNF-α and NF-KB gene expression, but NOS2 was significantly higher (P<0.01) and NOS3 lower (P<0.001), versus the control group (Table I).

In the CRF animals, in both the kidney and the heart, IL-2 gene expression was higher (P<0.05) that in the control rats (Fig. 2A3 and 2B3, respectively), without no further significant changes on all the other markers (Table I and II, respectively).

In the rats under rhEPO treatment with CRF (CRF+rhEPO group), IL-2 gene overexpression found in the CRF animals was prevented in the kidney (P<0.01) and a trend to identical variation was found in the heart (Fig. 2A3 and 2B3, respectively). Apart from those changes, a trend to lower values was found for the kidney TNF-α gene expression and higher for heart NF-KB, together with a reduction of NOS3 in the kidney (P<0.01) versus the CRF group (Table I and II).

DISCUSSION

The introduction of recombinant human erythropoietin (rhEPO) therapy allowed a significant reduction of anemia-associated adverse effects, allowing for a prolonged life expectance in end-stage renal disease stages[4]. Apart the anemia correction, rhEPO therapy has been associated with positive beneficial effects on non-hematopoietic cells[7-9], which have been attributed to its anti-apoptotic, anti-inflammatory and antioxidant actions, that underlies the cardio and neuro protection in other conditions[20,21]. Functional EPO receptors have been found to be expressed in several tissues, included the cardiovascular system, namely in endothelial cells and cardiomyocytes[22,23] and EPO is a key player in a broad variety of processes in cardiovascular pathophysiology, including apoptosis, cell proliferation, ischaemia and the nitric oxide pathway[7]. An increase in cardiac systolic function has been observed in patients with chronic heart failure treated with EPO. Other beneficial effects appear to be related to the pro-angiogenic properties on endothelial cells and could be useful for treatment of ischemic heart disease[24]. These findings suggest that rhEPO could provide potential therapeutic benefits in the management of cardiovascular diseases beyond anaemia correction. Nevertheless, although enhanced EPO synthesis is viewed as an appropriate compensatory mecha-

nism in the cardio-renal syndrome, excessive EPO synthesis in the advanced stages of both the CRF and CHF appears to be predictive of higher mortality[10]. In this context, earlier rhEPO therapy in anaemic CKD patients might have a positive cardio--renal impact, such as previously reported in ischemic injury, contributing to organ protection/ regeneration[23-26]. However, the impact of early rhEPO use, in stages of yet moderate renal failure and low cardiac deterioration, remains poorly investigated.

In order to further evaluate the effect of rhEPO on the cardio-renal axis, our group has previously characterized a rat model of moderate chronic renal failure induced by partial (3/4) nephrectomy, which demonstrated a moderate but maintained degree of CRF, together with transitory anemia and iron metabolism disturbances[11-13]. The remnant kidney presented a reasonable degree of functionality, mainly due to hypertrophic compensation, and there was several important cardiovascular modifications, including hypertension, tachycardia, dyslipidemia, erythropoietic disturbances, sympathetic activation, proliferation, angiogenesis and oxidative stress, which are features seen in CKD patients[11-13]. The use of rhEPO in that model showed important renal and cardio effects, including prevention of tachycardia, catecholamines increment and dyslipidemia, together with a notorious pro-proliferative action on the remnant kidney and on the heart tissue[12,13]. Considering that EPO interaction with its receptor leads to activation of several important pathways related with apoptosis, proliferation, angiogenesis, inflammation and lesion/stress[15-17,21], we hypothesize that the putative protective effect of early rhEPO use might be linked with adaptations on kidney and heart gene expression.

In our study, rhEPO treatment, per se, without CRF, showed a trend to higher EPO gene expression and a significant downregulation of EPO-R in the kidney, which was accompanied by overexpression of EPO and EPO-R gene in the heart. In the group with CRF, rhEPO treatment did not change EPO and EPO-R expression in the kidney, but a trend to higher values of both genes in the heart was found. This data suggest that rhEPO use, in this circumstances of moderate CRF, might produce a relevant extra--renal impact, namely at the level of cardiac tissue, as previously suggested by us using this model, as well as by others in other conditions[6,27].

Our study also showed that both the kidney and the heart from the rats with CRF presented a pro-apoptotic profile, viewed by the significantly increased ratio of Bax/Bcl2 expression in both tissues. This pattern was accompanied by the absence of other significant changes, including on gene expression of the par Fas-Faslg and on caspases 3 and 9. Recombinant erythropoietin use in these animals promoted prevention of apoptosis in both the remnant kidney and the heart, due to the significant prevention of Bax/Bcl2 increment induced by renal failure. This effect was previously documented for rhEPO use in other situations, in both the kidney[28-31] and in the heart, due to effect on cardiac myocytes[32-34], but was not previously evaluated on a moderate stage of chronic renal failure. A

similar preventive use, by the pleiotropic action, including mechanisms of anti-apoptotic action, was recommended by Bernhardt and Eckardt (2008) as protective against development of acute kidney injury in critically ill patients at higher risk of acute nephropathy[35].

Chronic kidney disease is associated with inflammatory mechanisms and is present in CKD patients, viewed by increment in the synthesis of acute phase proteins, such as C-reactive protein (CRP), a prominent marker of inflammatory response in the general population and in chronic kidney disease (CKD) patients, which was reported elevated in hemodialyzed patients[36-38]. Chronic inflammation is also associated with atherosclerotic cardiovascular disease (CVD) and the high rate of morbidity and mortality observed in haemodialyzed patients[39,40] could reflect the inflammatory process in CKD patients favouring CVD events. rHEPO has been shown a pleiotropic action[41-43], which includes an anti-inflammatory activity already reported on other conditions, including in cardiomyocytes with hypoxia/reoxygenation injury, in the brain, as well as in the kidney[44-48]. In our study, CRF was not associated with significant changes on the inflammatory markers, viewed by normal gene profile of both TNF-α and NF-kβ in both the remnant kidney and in the heart, which is in agreement with our previous assays in serum markers of inflammation[11-13]. However, the IL-2 gene overexpression in both tissues was prevented with rhEPO, suggesting an inhibitory effect on T-cell induced IL-2 production. Previous studies on CKD patients have shown serum IL-2 increment when compared with controls[49,50], an effect which was reduced by rhEPO treatment, demonstrating that rhEPO has an immunomodulatory action. Our data reinforces this notion, demonstrating that the rhEPO effect is due to downregulation of CRF induced IL-2 gene overexpression in the remnant kidney and in the heart on a moderate model of CRF.

Erythropoietin (EPO) is a hormone regulating the proliferation and differentiation of erythroid precursor cells, which have been demonstrating pro-proliferative and pro-angiogenic activity[24,51]. In our model of moderate CRF, kidney and heart gene expression of TGF-β1 and VEGF, which are well-recognized markers of proliferation and angiogenesis, respectively, was unchanged, thus demonstrating that other pathways should play a role on the pro-proliferative profile encountered in this model, previously demonstrated by us in his model by the increment in the trophism of both tissues, as well as by the increased levels of serum TGF-β1[13]. rhEPO treatment, accordingly, did not modify the expression of both genes in those tissues of the CRF rats. However, rhEPO was able to exacerbate the effect of chronic renal failure on PCNA expression in the remnant kidney and in the heart, thus demonstrating a proliferative action associated with DNA synthesis and repair, which are known functions of PCNA[52,53], thus suggesting a regenerative function of rhEPO on both tissues. Similar effect was reported in a model of acute renal failure induced by cisplatin in the rat[54], as well in the liver after resection in the rat[55], but was not previously documented nei-

ther on remnant kidney of moderate chronic renal failure nor on the corresponding heart.

Our study also assessed the influence of rhEPO treatment on kidney and heart gene expression of cytochrome c, viewed as a marker of mitochondrial injury, as well as on the expression of NO synthase 2 and 3, which are the key enzymes in inducible and constitutive NO synthesis and which participate in oxidative stress under unimpaired circumstances. Erythropoietin as been indicated as an antioxidant tissue-protective cytokine[56,57]. In our model of moderate CRF, both cytochrome c and NO synthases gene expression were unchanged in the remnant kidney and in the heart, suggesting that both tissues were in an earlier stage of oxidative lesion. rhEPO treatment, per se, without CRF, showed interesting effects on the kidney, including an increment of NOS2 gene expression, a complementary reduction of NOS3 and a significant augment of cytochrome c. Previous studies showed the influence of rhEPO on vascular nitric oxide synthase expression and activity[58,59], as well as in cytochrome c synthesis[60]. However, rhEPO use in the CRF animals has prevented the trend to increased kidney NOS3 gene expression in the CRF group, without any further effects in the CRF rats for NOS2 or NOS3 in the kidney or the heart, which is in agreement with the absence of effect found by other group in rats with chronic renal failure[61].

In conclusion, in this model of moderate chronic renal failure, rhEPO treatment showed important non-hematopoietic effects, expressed mainly by the anti-apoptotic and the pro-proliferative action on the kidney and on the heart. This data suggest that early rhEPO in moderate stages of CRF, before critical lesion of the tissues, might have further benefits.

Declaration of interest: The authors report no conflict of interest.

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COMPLETE BLOOD CELL COUNT AND RETINAL VESSEL DIAMETERS.

Klein BE, Klein R, Myers CE, Lee KE[1].

OBJECTIVE: To examine the cross-sectional associations of components of the complete blood cell count with retinal vessel diameters.

METHODS: The data are from the baseline examination of the Beaver Dam Eye Study cohort (n = 4730) from March 1, 1988, to September 14, 1990. Blood pressure was measured, a medical history including questions on cigarette smoking was obtained, and fundus photographs centered on the optic disc were taken and digitized. Retinal arteriole and venule diameters were measured using computer-assisted software. The central retinal arteriole equivalent and central retinal venule equivalent were computed. A complete blood cell count was done.

RESULTS: In age- and sex-adjusted analyses, red blood cell count, hemoglobin level, hematocrit, and white blood cell count were all statistically significantly associated with central retinal venule equivalent and central retinal arteriole equivalent, while platelet count was associated only with central retinal venule equivalent. These relationships persisted in more fully adjusted models, except platelet count became statistically significantly associated with both central retinal arteriole equivalent and central retinal venule equivalent.

CONCLUSIONS: Blood components as measured in a complete blood cell count are significant correlates of retinal vessel diameters and should be considered in analyses where retinal blood vessel diameters are outcomes. **[Arch Ophthalmol 2011; 129(4):490-497]**

PMID:21482874

[1] Department of Ophthalmology and Visual Sciences, School of Medicine and Public Health, University of Wisconsin-Madison, 610 N Walnut Street, Madison, WI 53726-2336, USA. kleinb@epi.ophth.wisc.edu

EFFECT OF AIR TRAVEL ON EXERCISE-INDUCED COAGULATORY AND FIBRINOLYTIC ACTIVATION IN MARATHON RUNNERS.

Parker B, Augeri A, Capizzi J, Troyanos C, Kriz P, D'Hemecourt P, Thompson P [1]

OBJECTIVE: Air travel and exercise change hemostatic parameters. This study investigated the effect of air travel on exercise-induced coagulation and fibrinolysis in endurance athletes.

PARTICIPANTS: Forty-one adults were divided into travel (T: 23 participants, living >4-hour plane flight from Boston) and nontravel (C: 18 participants, living <2-hour car trip from Boston) groups. INDEPENDENT VARIABLES: Age, anthropometrics, vital signs, training mileage, and finishing time were collected. **SETTING:** The 114th Boston Marathon (April 19, 2010).

MAIN OUTCOME MEASURES: Subjects provided venous blood samples the day before (PRE), immediately after (FINISH), and the day following the marathon after returning home (POST). Blood was analyzed for thrombin-antithrombin complex (TAT), tissue plasminogen activator (t-PA), hematocrit (Hct), and the presence of Factor V Leiden R506Q mutation.

RESULTS: Thrombin-antithrombin complex increased more in T subjects in PRE to FINISH samples (5.0 ± 4.0 to 12.9 ± 15.6 µg/L) than in C subjects (4.0 ± 1.2 to 6.1 ± 1.2 µg/L; P = 0.02 for comparison). The t-PA increased in both the T (5.4 ± 2.3 to 25.1 ± 12.2 ng/mL) and C (5.6 ± 2.0 to 27.7 ± 11.3 ng/mL) groups in PRE to FINISH samples, and this response did not differ between groups (P = 0.23 for comparison). Both groups exhibited similar t-PA and TAT values at POST that were not different than PRE (all P > 0.35). Age was related to the FINISH TAT values in T (r = 0.19; P = 0.04) but not in C (r = 0.03; P = 0.53) subjects.

CONCLUSIONS: Results suggest that the combination of air travel and marathon running induces an acute hypercoaguable state; this hemostatic imbalance is exaggerated with increasing age. [**Clin J Sport Med 2011; 21(2):126-130].**

PMID:21358503

[1] Henry Low Heart Center, Department of Cardiology, Hartford Hospital, Hartford, CT 06102, USA. bparker03@harthosp.org

16.º CONGRESSO INTERNACIONAL DA SOCIEDADE EUROPEIA PARA A HEMOREOLOGIA CLÍNICA E MICROCIRCULAÇÃO (ESCHM)

EXTRACTO DA PARTICIPAÇÃO PORTUGUESA

COMUNICAÇÃO LIVRE

RED BLOOD CELL AS A LINK BETWEEN BASIC AND CLINICAL RESEARCH

C Saldanha[1]

The normal metabolic, biorheological and membrane erythrocyte properties allow it to pass through the capillaries at microcirculation where oxygen, nitric oxide (NO) and carbon dioxide gas exchange occur between the red blood cell (RBCs) and tissue cells.

The characterization and evaluation of known RBCs properties has been done in basic and clinical research studies. Associations between several RBCs biochemical or hemorheological parameters and the degree of disease or its progression have been

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reported. In spite several mechanisms and signal transduction pathways had been described extensively, the cross talk between RBCs and the others blood and endothelial cells in both physiological and pathophysiological situations is still to be understand.

Examples of cross-sectional and longitudinal clinical studies done in vascular and metabolic disease like acute myocardial infarction, glaucoma, renal insufficiency and diabetes where erythrocyte parameters have been assessed and prognostic value evaluated were presented. Furthermore, examples of basic experimental studies were recall, among various done with erythrocytes obtained from either healthy donors or patients with vascular disease such as stroke, glaucoma and arterial hypertension. The description of these studies, among many others, point out the erythrocyte as a link between basic and clinical research.

PAINEIS

EVIDENCE THAT THE DEGREE OF BAND 3 PHOSPHORYLATION MODULATES HUMAN ERYTHROCYTES NITRIC OXIDE EFFLUX – IN VITRO MODEL OF HYPERFIBRINOGENEMIA

Lopes de Almeida JP[1,2], *Freitas-Santos T*[2], *Saldanha C*[1,2]

ABSTRACT

Recent evidence has shown that plasma fibrinogen, a major cardiovascular risk factor, interacts with the erythrocyte membrane and acts to influence blood flow via erythrocyte nitric oxide (NO) modulation. In the present pioneer *in-vitro* study, whole blood samples were harvested from healthy subjects and aliquots were incubated in the absence (control aliquots) and presence of fibrinogen at different degrees of band 3 phosphorylation, and the levels of NO, nitrite, nitrate and S-nitroglutathione (GSNO) were determined.

Hyperfibrinogenemia interferes with erythrocyte NO mobilization without changing its efflux in a way that seems to be independent of the degree of band3 phosphorylation. Higher levels of nitrite, nitrate and GSNO were documented ($p<0.05$). However, the mechanisms by which fibrinogen signalling modulates erythrocyte function remain to be clarified and are currently under study.

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[2] Institute of Biochemistry, Institute of Molecular Medicine, University of Lisbon Medical School, Av. Prof. Egas Moniz, 1649-028 Lisbon, Portugal

Evidence That The Degree Of Band 3 Phosphorylation Modulates Human Erythrocytes Nitric Oxide Efflux

In Vitro Model Of Hyperfibrinogenemia

ESCHM 2011 Munich, GERMANY JUNE 18th - JUNE 21st 2011

J. Pedro Almeida, MD; **T. Freitas-Santos; Carlota Saldanha**, PhD

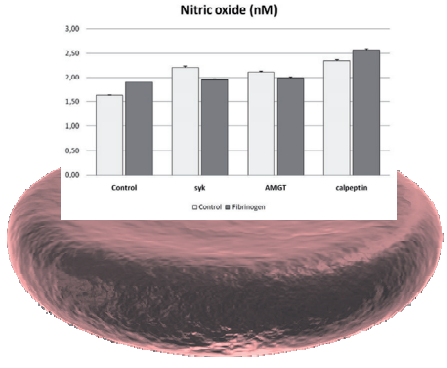
Institute of Biochemistry, Institute of Molecular Medicine (IMM), University of Lisbon Medical School, Santa Maria Hospital, Lisbon – Portugal

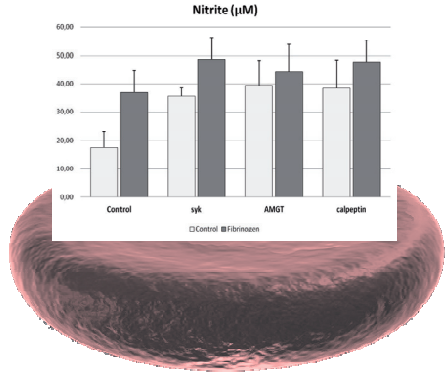
E-mail: jpedro.gla@gmail.com

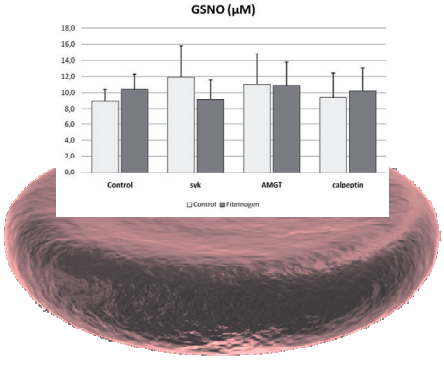
Background. Experimental evidence has shown that plasma fibrinogen plays a key role as a major cardiovascular risk factor, acting directly to trigger erythrocyte aggregation in occlusive vascular disease. However, due to the complex and hitherto unclear interaction between fibrinogen and the erythrocyte membrane, no study has yet evaluated the effects of fibrinogen, under and above physiological range values, on the erythrocyte nitric oxide (NO) mobilization. However, no work has yet assessed the effect of high levels of plasma fibrinogen on the erythrocyte nitric oxide metabolism (nitrite, nitrate) and mobilization (S-nitroso-glutathione) on the dependence of the degree of band 3 phosphorylation, which was the purpose of this study.

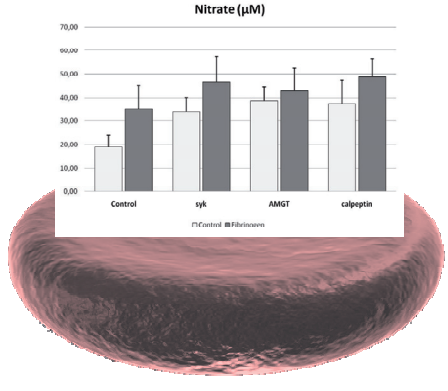
Methods. In this *in-vitro* study, whole blood samples were harvested from healthy subjects and erythrocyte suspensions were incubated in the absence (control aliquots) and presence of different fibrinogen concentrations. The levels of NO were determined by an amperometric method, nitrite, nitrate and S-nitroglutathione (GSNO) were measured using the spectrophotometric Griess reaction.

Results. This is the first study to explore an association between fibrinogen and the degree of band 3 phosphorylation on the modulation of erythrocyte nitric oxide efflux and mobilization. Hyperfibrinogenemia interferes with erythrocyte NO mobilization without changing its efflux in a way that seems to be independent of the degree of band3 phosphorylation. Higher levels of nitrite, nitrate and GSNO were documented ($p<0.05$).









Conclusions. This study gains new insights into an unknown mechanism by which fibrinogen modulates the erythrocyte capacity to supply nitric oxide. The precise mechanisms by which plasma fibrinogen interacts with the erythrocyte membrane to mobilize NO from store molecules into its oxidative metabolites (and vice-versa) are still vague. Further studies are compulsory to deepen this topic of major impact in human inflammatory conditions. Also, increased erythrocyte GSNO levels may be associated with platelet NO metabolism, its activation status and hypotension, which may be extremely relevant in the clinical setting as biomarkers.

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA E DO ENSINO SUPERIOR Portugal

HEMORHEOLOGICAL AND HAEMOSTATIC PROFILES IN HYPERTENSIVE SUBJECTS

ESCHM 2011 Munich, GERMANY JUNE 18th - JUNE 21st 2011

Lopes de Almeida JP, Freitas T, Braz-Nogueira J, Martins-Silva J, Saldanha C

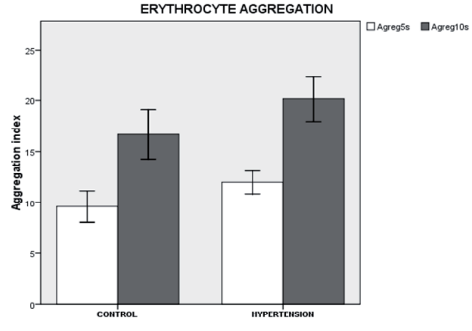
Institute of Biochemistry, Institute of Molecular Medicine (IMM), University of Lisbon Medical School, Santa Maria Hospital, Portugal

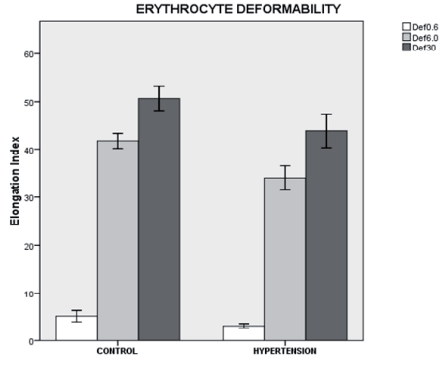
E-mail: jpedro.gla@gmail.com

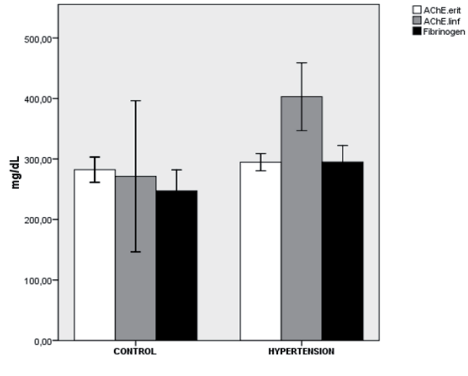
Background. Hemorheological disturbances in hypertension are the outcome of changes in red cell characteristics, their interaction in the presence of fibrinogen, and the magnitude and distribution of flow forces. The higher susceptibility of hypertensives to the development of atherosclerotic disorders and strokes could be due to the presence of a hyperreactive coagulation system coupled with changed endothelial, rheological and platelet functions.

Methods. The aim of this study was to investigate the hemorheological profile in hypertensive subjects (n=15), compared with a normotensive group (n=30). The following variables were measured: erythrocyte aggregation, erythrocyte deformability, blood viscosity, membrane fluidity, fibrinogen and erythrocyte and lymphocyte acetylcholinesterases.

Results. Our results showed that the hypertensive group sustained decreased erythrocyte deformability, increased aggregation, higher levels of fibrinogen and of erythrocyte and lymphocyte acetylcholinesterases ($p<0.05$), as well as decreased nitric oxide and increased blood viscosity (NS).







Conclusions. This study gains new insights into an unknown mechanism by which fibrinogen modulates the erythrocyte capacity to supply nitric oxide. The precise mechanisms by which plasma fibrinogen interacts with the erythrocyte membrane to mobilize NO from store molecules into its oxidative metabolites (and vice-versa) are still vague. Further studies are compulsory to deepen this topic of major impact in human inflammatory conditions. Also, increased erythrocyte GSNO levels may be associated with platelet NO metabolism, its activation status and hypotension, which may be extremely relevant in the clinical setting as biomarkers.

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA E DO ENSINO SUPERIOR Portugal

MEDALHA FARHAEUS

No âmbito do Congresso foi atribuída, como é tradição, a medalha Farhaeus a um cientista que mais se tenha distinguido por trabalhos e participação no campo da Hemorreologia e Microcir culação. A distinção coube, desta feita, ao Professor W Reinhart, presidente da Sociedade Suíça, que havia sido o presidente e organizador do 15.º Congresso da ESCHM.

Professor W. Reinhart

PRÓXIMAS REUNIÕES INTERNACIONAIS SOBRE MICROCIRCULAÇÃO

CONGRESSO DA SOCIEDADE EUROPEIA DE MICROCIRCULAÇÃO EM CONJUNTO COM A SOCIEDADE ALEMÃ DE MICROCIRCULAÇÃO E BIOLOGIA VASCULAR

Welcome

Welcome to the Joint Meeting of the European Society for Microcirculation (ESM) and the German Society of Microcirculation and Vascular Biology (GfMVB) from 13-16 October, 2011 at the Ludwig-Maximilians-University in Munich, Germany.

8th Asian Congress for Microcirculation – 26-28 October, 2011, Bangkok, Thailand, http://www.worldmicrocirc.org/)

10th World Congress for Microcirculation – 2015, Beijing, China**.**

APRESENTAÇÃO DOS MEMBROS DOS ORGÃOS SOCIAIS DA SPHM (BIÉNIO 2009-2011)

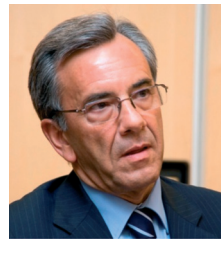
DIRECÇÃO

Presidente – **Prof.ª Doutora Carlota Saldanha**

Professora Associada c/ Agregação da Faculdade de Medicina da Universidade de Lisboa;
PI Unidade de Biologia Microvascular e Inflamação -Instituto de Medicina Molecular da Faculdade de Medicina da Universidade de Lisboa.
Presidente da Sociedade Portuguesa de Hemorreologia e Microcirculação.
Membro do Grupo Estratégico da European Society of Microcirculation.
Membro do Conselho Editorial do Clinical Hemorheology and Microcirculation.

Vice-Presidentes:

Prof. Doutor Henrique Luz Rodrigues



Professor de Farmacologia da Faculdade de Medicina da Universidade de Lisboa
Assistente de Nefrologia do Hospital de Santa Maria
Presidente do Colégio de Farmacologia Clínica da Ordem dos Médicos.

Dr. José António Pereira Albino

Licenciado em Medicina pela Universidade Nova de Lisboa em 1978
Assistente Hospitalar de Angiologia e Cirurgia Vascular (H. St Marta) em 1989
Consultor de Angiologia e Cirurgia Vascular em 1990
Chefe de Serviço de Angiologia e Cirurgia Vascular do H. St Marta em 2000
Director do Serviço de Angiologia e Cirurgia Vascular do H. Pulido Valente (actualmente, Serviço de Cirurgia Vascular II do CHLN) desde 2004
Director do Bloco Operatório do H. Pulido Valente desde 2006
Adjunto da Direcção Clinica do CHLN desde 2008
Professor convidado de Cirurgia da Faculdade de Ciências Médicas de Lisboa desde 2008
Membro de várias Sociedades Cientificas Nacionais e Internacionais, tendo exercido vários cargos directivos nessas sociedades e no Colégio da Especialidade da Ordem dos Médicos

Secretário-Geral – **Flávio Nelson Fernandes Reis** (freis@fmed.uc.pt)

Grau Académico:
Doutoramento em Ciências Biomédicas pela Faculdade de Medicina da Universidade de Coimbra.
Actividade e interesses actuais em investigação:
Investigador Auxiliar da FMUC.
Farmacologia e Terapêutica, clínica e experimental; Doenças cardiometabólicas; Insuficiência renal e alterações hemorreológicas; Oncologia Urológica.
Co-autoria de 130 trabalhos publicados e de 15 Premiados.
Revisor Convidado de 35 artigos em revistas internacionais e Revisor residente da Acta Urológica.
Orientador/Co-orientador de 4 teses de Doutoramento e de 28 de Mestrado em áreas Biomédicas.
Actividade anterior:
Docente da FMUC, Farmacologia e Terapêutica Geral.
Membro da Assembleia de Representantes e Conselho Directivo da FMUC e do Senado da UC.
Cargos actuais:
Membro da Comissão de Gestão do Laboratório de Comportamento e Experimentação Animal da FMUC e da Comissão de Avaliação para o SIADAP.

Tesoureiro – **Prof. Doutor Henrique Sobral do Rosário**

Educação
2009 – Doutoramento em Medicina (Bioquímica) pela Universidade de Lisboa
1995 – Licenciatura em Medicina, Faculdade de Medicina daUniversidade de Lisboa
Posição actual
2010 – Professor Auxiliar, Instituto de Bioquímica, Faculdade de Medicina da Universidade de Lisboa
Investigador, Unidade de Biologia Microvascular e Inflamação, Instituto de Medicina Molecular
Publicações científicas
• Co-autoria em 16 artigos científicos publicados em revistas internacionais indexadas.
• Co-autoria em 51 apresentações em reuniões científicas internacionais.
• Co-autoria em 2 capítulos de livros.
Prémios
• Prémio Pfizer para Jovens Investigadores 1999, atribuído pela Sociedde de Ciências Médicas de Lisboa
Interesses em investigação
• Mecanismos moleculares de inflamação.
• Imunidade inata.
• Interacção leucócito-endotélio.
• Inflamação em aterosclerose.
• Microscopia intravital da inflamação.
Correspondência:
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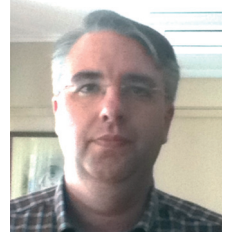
Prof. Doutor José Luís Bliebernicht Ducla Soares

Professor catedrático de Medicina, Faculdade de Medicina da Universidade de Lisboa
Serviço de Medicina ID, Hospital de Santa Maria, Lisboa

Dr. Jorge Lima (jorgeramoslima@sapo.pt)

- Licenciatura em Medicina (Faculdade de Medicina da Universidade de Lisboa) 1995
- Assistente Convidado de Bioquímica Fisiológica da FML (1993-2004)
- Especialista em Ginecologia e Obstetrícia da Maternidade Dr. Alfredo da Costa (2004-2005)
- Assistente Hospitalar de Ginecologia e Obstetrícia do Hospital de Dona Estefânia (2005-2006)
- Especialista em Ginecologia e Obstetrícia do Hospital CUF Descobertas, desde 2004, sendo responsável pela Consulta de Trombofilias

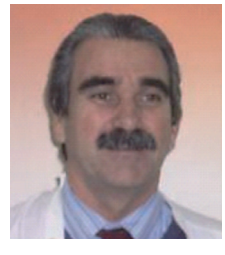
Dr. Luís Sargento



Luis José Morais Sargento, natural de Lisboa, a 15 de Outubro de 1970.
Assistente Hospitalar de Cardiologia no Centro Hospitalar Lisboa Norte.
Fellow da Sociedade Europeia de Cardiologia.
Áreas de interesse actual: insuficiência cardíaca e ecocardiografia.
Vinte artigos publicados em revistas com peer-review (artigo de maior cotação ISI 4,985) e cerca de 200 comunicações em Congressos Nacionais e Internacionais.

ASSEMBLEIA GERAL

Presidente – **Prof. Doutor José Manuel Braz Nogueira**



Professor Catedrático de Medicina da Faculdade de Medicina de Lisboa (FML).
Director do Serviço de Medicina I do Hosp. de St.ª Maria/CHLN.
Coordenador da Área de Medicina I, Regente de Medicina Interna I do 4.º ano e Coordenador do Estagio de Medicina (6.º ano) do Mestrado Integrado em Medicina da FML.
Membro do Conselho Cientifico da FML.
Especialista Europeu de Hipertensão Arterial.
Vice-Presidente da Direcção da Sociedade Portuguesa de Hemorreologia e Microcirculação (2007-2009).
Editor-Chefe da Revista Portuguesa de Hipertensão e Risco Cardiovascular

Primeiro Secretário – **Prof. Doutor Luís Mendes Pedro**

Nome: Luís Alberto da Cunha Mendes Pedro
Data de nascimento: 24 de Setembro de 1963
Cargos/funções actuais:
• Professor Associado da Faculdade de Medicina de Lisboa (Ciências Cirúrgicas- Cirurgia Vascular).
• Assistente Hospitalar Graduado de Cirurgia Vascular no Hospital de Santa Maria
• Cirurgião Vascular do Instituto Cardiovascular de Lisboa

Segundo Secretário – **Dr. Miguel Frederico Leal Galvão**

Licenciado em Medicina pela Faculdade de Ciências Médicas da UNL, tem o Grau de Chefe de Serviço/ Assistente Graduado Sénior.
Director do Serviço de Imuno-Hemoterapia do Hospital de Santa Maria (HSM) desde 1990 é actualmente Director do Serviço de Imuno-Hemoterapia no Centro Hospitalar de Lisboa Norte, EPE, que compreende no HSM o Centro de Medicina Transfusional, o Centro Ambulatório de Imuno-Hemoterapia, o Centro de Coagulopatias Congénitas e o Centro de Terapia Celular e um pólo no Hospital Pulido Valente.
Foi nomeado em 2004 Director do Departamento de Meios Complementares de Terapêutica do HSM.
É Especialista em Imuno-Hemoterapia pela Ordem dos Médicos.
Tem a Competência em Gestão dos Serviços de Saúde da Ordem dos Médicos.
É "Foundation Fellow in Haematology and Transfusion Medicine" pelo European Board of Medical Biopathology.

Primeiro Secretário Suplente – **Dr.ª Ana Santos Silva Herdade**

Licenciada em Química Aplicada/Biotecnologia pela Faculdade de Ciências e Tecnologia (UNL).
Assistente no Instituto de Bioquímica da Faculdade de Medicina de Lisboa
Investigador da Unidade de Biologia Microvascular e Inflamação do Instituto de Medicina Molecular

Segundo Secretário Suplente – ***Dr. Paulo Pereira da Silva***

CONSELHO FISCAL

Presidente – **Prof. Doutor João Eurico Fonseca**

João Eurico Fonseca, MD, PhD, is Head of the Rheumatology Research Unit at the Instituto de Medicina Molecular, and Professor of Rheumatology and Biomedical Engineering, both part of the Faculty of Medicine, University of Lisbon, Portugal.
He is also Head of the Day Care Unit of the Rheumatology and Bone Metabolic Diseases Department at the Hospital de Santa Maria, Lisbon, and Vice President of the Portuguese Society of Rheumatology.

Primeiro Vogal – **Dr.ª Maria Helena Baptista Manso Ribeiro**

Licenciatura em Medicina pela Universidade Clássica de Lisboa em 1977, com 16 valores.
Assistente de Bioquímica da Faculdade de Medicina de Lisboa até 1985, tendo colaborado em vários projectos de investigação.
Especialista de Medicina Física e de Reabilitação.
Chefe de Serviço do quadro do Hospital de Tomar.
Foi Adjunta do Director Clínico; Presidente da Comissão de Farmácia e Terapêutica e da Comissão de Ética.
Integrou o Departamento de Formação e Comissão da Qualidade.
É Directora do Departamento de Meios Auxiliares de Terapêutica do Centro Hospitalar do Médio Tejo, que engloba os Serviços de Imunohemoterapia e os Serviços de Reabilitação dos Hospitais de Tomar, Torres Novas e Abrantes.

Segundo Vogal – **Prof. Doutor Carlos Manuel dos Santos Moreira**

Licenciado em Medicina em 1978 pela Faculdade de Medicina de Lisboa.
Curso Mestrado em Gestão em Saúde na Faculdade de Medicina de Lisboa.
Curso mestrado em Educação Médica pela Faculdade Católica de Lisboa.
Tese de doutoramento em Medicina na Faculdade de Medicina de Lisboa – "Hipertensão de Bata Branca" em 2009.
Especialista em Medicina Interna em 1995 exame conjunto da Ordem dos Médicos e Ministério da Saúde.
Especialista europeu em Hipertensão arterial em 2006 pela Sociedade Europeia de Hipertensão.
Competência em Gestão de Serviços de Saúde pela Ordem dos Médicos em 2003.

REGRAS DE PUBLICAÇÃO / INSTRUCTIONS TO AUTHORS

CONVITE

A Sociedade Portuguesa de Hemorreologia e Microcirculação (SPHM) aceita para publicação no seu BOLETIM artigos de curta extensão. O Boletim é editado quatro vezes por ano em formato de papel e electrónico (www.hemorreologia.com), sendo distribuído gratuitamente a individualidades e instituições científicas e culturais.

INSTRUÇÕES

1. Todos os textos enviados para publicação estão sujeitos a apreciação editorial e aprovação. A decisão é baseada no mérito científico e cultural dos trabalhos.

2. São aceites somente os trabalhos preparados em versão óptica (*PDF* ou *Microsoft Word*).

3. Os textos devem ser redigidos em Português ou Inglês.

4. Os manuscritos com o pedido de publicação devem ser enviados por *e-mail* ao Editor (carlotasaldanha@fm.ul.pt).

– Comunicações Originais (artigos curtos) – Os textos serão considerado para publicação rápida, com a seguinte estrutura: Sumário (50-70 palavras), Introdução, Material e Métodos, Resultados, Discussão e Conclusões. O(s) autor(es) são estimulados a englobar em conjunto os resultados, discussão e conclusões.
(Extensão máxima do texto: 5 a 6 páginas a um espaço (letra de corpo 11), incluindo figuras tabelas e quadros(e respectivas legendas),agradecimentos e até 30 referências bibliográficas).

– Artigos de Revisão – O BOLETIM terá a maior satisfação em acolher curtas revisões sobre assuntos de particular interesse, no âmbito da Hemorreologia, Microcirculação ou assuntos de âmbito médico ou de outras áreas científicas afins, que sejam submetidos directamente para publicação ou mediante convite especial do Editor.
(Extensão máxima do texto:8 a 10 páginas (letra de corpo 11) incluindo figuras, tabelas, quadros, fotos (e respectivas legendas), agradecimentos e até 60 referências bibliográficas).

INVITATION

The Portuguese Society on Hemorheology and Microcirculation (Sociedade Portuguesa de Hemorreologia e Microcirculação, SPHM) is pleased to welcome short papers for publication in its BOLETIM. This publication, in paper and online (www.hemorreologia.com), is distributed four times a year free of charge to the members of the Society.

INSTRUCTIONS

1. All submitted manuscripts are subjected to editorial review and approval. The decision to publish is dependent on the scientific and cultural merit of the papers.

2. Only contributions prepared and submitted as optic version (*PDF* or *Microsoft Word*), will be accepted.

3. Texts must be written in Portuguese or in English.

4. All scientific contributions, including manuscript submission and further correspondence should be addressed by *email* to the Editor (carlotasaldanha@fm.ul.pt)

– Original Communications – Manuscripts may be considered for rapid processing as short communications. All manuscripts should be arranged in the following sections: Abstract (50-70 words), Introduction, Material and Methods, Results, Discussion, Acknowledgements and References. The author(s) may combine some of the sections normally included in a full paper, namely the results, discussion and conclusions.
(Maximum communication length – 5-6 single spaced typed pages, including figures, tables, legends, acknowledgments and up to 30 references).

– Short Reviews – The BOLETIM will publish reviews on subjects of particular interest in its field, either following a special invitation or a submission by the author, and in the latter case only after approval by an Editorial Board member. Further information can be obtained from the editor.
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