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Níveis Séricos de Vitamina D em Pacientes em Morte Encefálica

Porto Alegre, 2017

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RESUMO

A vitamina D é uma vitamina lipossolúvel sintetizada na pele em resposta à exposição solar e que regula o metabolismo do cálcio. Nos últimos anos, com o avanço de estudos moleculares e genéticos, observou-se que a vitamina D tem uma gama de efeitos maior do que se pensava. Esses efeitos, denominados efeitos pleiotrópicos, incluem a potencialização da ação antimicrobiana e alterações do perfil inflamatório, além de efeitos cardioprotetores e imunomoduladores. As concentrações de vitamina D diminuem durante doenças agudas que se apresentam com aumento de atividade inflamatória. Além disso, concentrações aumentadas de marcadores inflamatórios, como TNF-α ou proteína C reativa, estão inversamente correlacionadas com níveis de vitamina D, sugerindo que a inflamação seja um fator relacionado a baixos níveis de vitamina D em doenças não ósseas. A morte encefálica (ME) é uma síndrome que cursa com intensa atividade inflamatória, caracterizada pelo aumento da concentração plasmática de citocinas. A ME está associada a piores desfechos dos órgãos transplantados quando comparados aos órgãos provenientes de doadores vivos, estando relacionada à maior incidência de disfunção primária dos enxertos. Portanto, o objetivo deste estudo foi comparar os níveis de vitamina D em pacientes em ME com os níveis de pacientes críticos sem diagnóstico de morte encefálica e definir se havia correlação entre níveis de vitamina D e marcadores inflamatórios em pacientes em ME. Demonstramos que não há diferença em relação aos níveis séricos de vitamina D entre os dois grupos. No entanto, em pacientes em ME, as citocinas IL-8, IL-10 e IFN-y apresentaram correlação positiva com níveis de vitamina D.

ABSTRACT

Vitamin D is a fat-soluble vitamin synthesized by the skin in response to sunlight exposure and regulates calcium metabolism. More recently, advances in molecular and genetic fields showed vitamin D to have a wider range of effects than previously thought. These so-called pleiotropic effects include changes in inflammatory profile, as well as cardioprotective and immunomodulatory effects. Vitamin D levels decrease during acute diseases with increased inflammatory activity. Besides, increased concentrations of inflammatory markers, such as tumor necrosis factor-α (TNF-α) or C-reactive protein, are inversely correlated with vitamin D levels, suggesting that an inflammatory process is involved in low levels of vitamin D in non-skeletal diseases. Brain death (BD) is a syndrome with an intense inflammatory activity characterized by the upregulation of plasma cytokines. BD has been associated with an increased risk of acute and chronic rejection and with a higher incidence of primary graft dysfunction. Therefore, the objective of this study was to evaluate vitamin D levels in patients with BD as compared to those from critically ill patients without BD and define if there were correlations between vitamin D levels and inflammatory markers in patients with BD. We demonstrated that serum levels of vitamin D did not differ between groups. However, IL-8, IL-10 and IFN-γ showed a moderate direct correlation with vitamin D levels in brain-dead patients.

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LISTA DE ABREVIATURAS

APC	*Antigen presenting cells*
BD	Brain death
BMI	Body mass index
FIPE	Fundo de Incentivo à Pesquisa e Ensino
HCPA	Hospital de Clínicas de Porto Alegre
ICU	Intensive care unit
IFN-γ	interferon--γ ou Interferon-γ
GLP-1	Glucagon-like peptide 1
IL-1β	Interleukin-1β
IL-2	Interleukin-2
IL-4	Interleukin-4
IL-6	Interleukin-6
IL-8	Interleucina-8 ou Interleukin-8
IL-10	Interleucina-10 ou Interleukin-10
IL-13	Interleukin-13
ME	Morte encefálica
MFI	Mean fluorescence intensity
SD	Standard deviation
TLR	Toll-like receptors
TNF-α	Tumor necrosis factor-α
UFRGS	Universidade Federal do Rio Grande do Sul
UTI	Unidade de tratamento intensivo
VDR	Receptor da vitamina D ou Vitamin D receptor
25(OH)D	25-hydroxyvitamina D ou 25-hydroxyvitamin D

SUMÁRIO

Esta dissertação de Mestrado será apresentada no formato exigido pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia. Ela será constituída de uma introdução em português e um artigo em inglês, este formatado conforme as exigências da respectiva revista médica à qual será submetido para avaliação e posterior publicação. O artigo em inglês desta dissertação é um artigo do tipo Artigo Original.

CAPÍTULO 1

INTRODUÇÃO

A vitamina D é uma vitamina lipossolúvel sintetizada na pele em resposta à exposição solar e tem um papel importante na regulação do metabolismo do cálcio.[1] O 7-dehidrocolesterol é convertido em colecalciferol (vitamina D3) na pele pela estimulação da radiação ultravioleta (Figura 1). A ingestão adequada de alimentos como ovos, peixes, laticínios e alguns cogumelos tem uma contribuição menor na oferta de vitamina D3 e do ergocalciferol (vitamina D2). As vitaminas D3 e D2 são convertidas em 25-hidroxivitamina D (25 (OH) vitamina D) por meio da 25-hidroxilase hepática e esta é convertida na sua forma ativa (1,25-hidroxivitamina D) pela 1-alfa-hidroxilase renal.[1,4] A 25 (OH) vitamina D mantém níveis séricos constantes e sua dosagem é representativa do estoque de vitamina D. A 25 (OH) vitamina D tem uma meia-vida de aproximadamente duas a três semanas. [1,4]

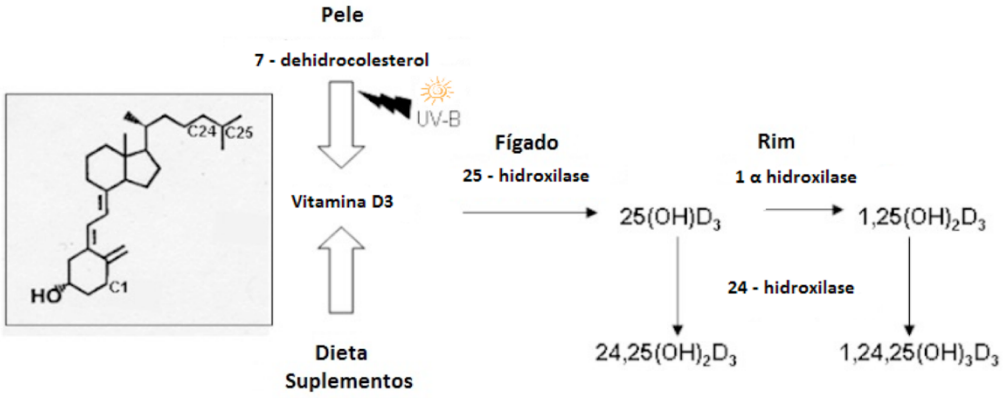

Figura 1 - Metabolismo da vitamina D. O 7-dehidrocolesterol isomeriza-se em colecalciferol na pele por meio da ação dos raios ultravioleta. Em seguida é transportado ao fígado, onde sofre a ação da 25-hidroxilase transformando-se em 25-hidroxivitamina D. Quando essa molécula chega ao rim, pode transformar-se na forma ativa ou inativa deste hormônio, por meio da 1-alfa-hidroxilase ou 24,25-hidroxilase, respectivamente.

Nos últimos anos, com o avanço de estudos moleculares e genéticos, observou-se que a vitamina D tem uma gama maior de efeitos do que se pensava anteriormente. Esses efeitos, denominados pleiotrópicos, incluem a potencialização da ação antimicrobiana e alterações do perfil inflamatório, além de efeitos cardioprotetores e imunomoduladores.[5,7] Foi demonstrado que a vitamina D têm

propriedades anti-inflamatórias e antiproliferativas, e sua deficiência tem sido associada ao desenvolvimento de doenças crônicas, câncer, doenças autoimunes e à maior mortalidade.[8] Esses efeitos pleiotrópicos da vitamina D podem estar relacionados à presença de receptores de vitamina D em vários órgãos e diferentes tipos celulares, além dos classicamente associados à regulação do metabolismo ósseo.[9,10]

A associação entre deficiência de vitamina D e desfechos adversos em pacientes críticos, como a maior mortalidade, tempo de internação, tempo de ventilação mecânica e infecções, ainda é conflitante.[11,13] Além disso, não se sabe se a deficiência de vitamina D no momento da internação em unidade de terapia intensiva (UTI) afeta a sobrevida de pacientes críticos ou se é apenas um marcador de maior gravidade da doença de base.[5] No entanto, existem evidências de que a doença crítica altera os níveis de vitamina D, com uma pequena queda observada nos seus níveis ao longo dos dias de internação em UTI.[14,15]

Diferentes lesões graves ao encéfalo podem resultar em morte encefálica (ME), que é definida como a cessação completa e irreversível das funções encefálicas e representa o processo final de isquemia rostro-caudal que culmina na herniação do tronco cerebral através do forame magno.[16] A ME constitui-se de uma síndrome clínica que causa desregulação homeostática, alterações na função endócrina e intensa atividade inflamatória, capazes de reduzir a tolerância dos órgãos à isquemia, levando a graves efeitos adversos sobre os desfechos dos órgãos transplantados.[17] Estudos experimentais demonstraram que a ME está associada a uma grande liberação de catecolaminas, resultando em alterações hormonais e bioquímicas.[18,19] O metabolismo celular é influenciado pela inflamação e é provável que as alterações metabólicas que ocorrem após a ME modulem a resposta inflamatória.[20,21] O transplante de órgãos é considerado o tratamento de eleição para várias doenças terminais que afetam rins, pâncreas, fígado, coração e pulmão.[22] Atualmente, a principal fonte de órgãos para transplante é o doador de órgãos em ME.[23] Por este motivo, o estudo dos fatores associados à resposta inflamatória desencadeada pela ME poderá resultar em um melhor entendimento dos mecanismos responsáveis pelos piores desfechos encontrados nos órgãos transplantados provenientes de doadores em ME.

Como a vitamina D tem propriedades anti-inflamatórias[24], seus níveis mais elevados no momento da ME poderiam resultar em atenuação da inflamação

tipicamente encontrada neste cenário clínico. Por outro lado, como as concentrações de vitamina D diminuem substancialmente durante doenças agudas que se apresentam com atividade inflamatória aumentada e falência de órgãos,[25,27] a ME poderia desencadear redução nos níveis séricos desta vitamina.

Frente ao exposto, esta dissertação tem dois objetivos:

- Determinar os níveis séricos de 25 (OH) vitamina D em pacientes em ME e compará-los aos níveis de pacientes críticos sem diagnóstico de morte encefálica;

- Avaliar se há correlação entre níveis séricos de 25 (OH) vitamina D e citocinas inflamatórias em pacientes criticamente doentes, com e sem ME.

REFERÊNCIAS

1. Holick MF. Vitamin D: Photobiology, metabolism, and clinical applications. In: de Groot LC, ed. Endocrinology. 1995;990-1011.

2. Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-281.

3. Biesalski HK. Vitamin D recommendations: beyond deficiency. Ann Nutr Metab. 2011;59:10-6.

4. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-1930.

5. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014;2:76-89.

6. Souberbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. Autoimmun Rev. 2010;9:709 -715.

7. Holick MF. Vitamin D deficiency in 2010: health benefits of vitamin D and & sunlight – a D-bate. Nat Rev Endocrinol. 2011;7:73-75.

8. Chu MP, Alagiakrishnan K, Sadowski C. The cure of ageing: vitamin D - magic or myth? Postgrad Med J. 2010;86:608–16.

9. Carthy EP, Yamashita W, Hsu A, Ooi BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. Hypertension. 1989;13:954–959.

10. Gysemans CA, Cardozo AK, Callewaert H, et al. 1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: Implications for prevention of diabetes in non-obese diabetic mice. Endocrinology. 2005;146:1956-1964

11. Matthews LR, Ahmed Y, Wilson KL, Griggs DD, Danner OK. Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive

care unit cost, and mortality rate in surgical intensive care unit patients. Am J Surg. 2012;204:37-43.

12. Arnson Y, Gringauz I, Itzhaky D, Amital H. Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients. QJM. 2012; 105:633-9.

13. Flynn L, Zimmerman LH, McNorton K, et al. Effects of vitamin D deficiency in critically ill surgical patients. Am J Surg. 2012;203:379-82.

14. Moraes RB, Friedman G, Wawrzeniak IC et al. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics. 2015;70(5):326-332.

15. Higgins DM, Wischmeyer PE, Queensland KM, Sillau SH, Sufit AJ, Heyland DK. Relationship of vitamin D deficiency to clinical outcomes in critically ill patients. JPEN J Parenter Enteral Nutr. 2012;36:713-20.

16. Wijdicks EFM. The landmark “Le Coma Dépassé”. In: Wijdicks EFM. Brain death. Philadelphia: Lippincott Williams & Wilkins; 2001.

17. Jassem W, Koo DD, Cerundolo L, Rela M, Heaton ND, Fuggle SV. Leukocyte infiltration and inflammatory antigen expression in cadaveric and living-donor livers before transplant. Transplantation. 2003;75(12):2001-7.

18. Novitzky D, Cooper DK, Rosendale JD, Kauffman HM. Hormonal therapy of the brain-dead organ donor: experimental and clinical studies. Transplantation. 2006;82(11):1396-401.

19. Takada M, Toyama H, Tanaka T, Suzuki Y, Kuroda Y. Augmentation of interleukin-10 in pancreatic islets after brain death. Transplant Proc. 2004;36:1543-6.

20. Barklin A. Systemic inflammation in the brain-dead organ donor. Acta Anesthesiol Scand. 2009;53:425-435.

21. Rech TH, Crispim D, Rheinheimer J, et al. Brain Death induced inflammatory activity in human pancreatite tissue: A case control study. Transplantation. 2014;97(2):212-219.

22. Rech TH, Rodrigues Filho, EM. Manuseio do Potencial Doador de Múltiplos Órgãos. Revista Brasileira de Terapia Intensiva. 2007;19(2):197-204.

23. Nagata H, Matsumoto S, Okitsu T, Iwanaga Y, et al. Procurement of the human pancreas for pancreatic islet transplantation from marginal cadaver donors. Transplantation. 2006;82(3):327-31.

24. Bellia A, Garcovich C, D`Adamo M, et al. Serum 25-hidroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern Emerg Med. 2013;8:33-40.

25. Van den Berghe G, Van Roosbroeck D, Vanhove P, et al. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab. 2003;88: 4623-32.

26. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360:1912-14.

27. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond). 2008;24:5-10.

CAPÍTULO 2

ARTIGO - ASSOCIATION BETWEEN VITAMIN D LEVELS AND INFLAMMATORY ACTIVITY IN BRAIN DEATH - Running title: Vitamin D and inflammatory activity in brain death

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Association between vitamin D levels and inflammatory activity in brain death

Running title: Vitamin D and inflammatory activity in brain death

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Key words: brain death, vitamin D, cytokines, inflammation.

ABSTRACT

Background and aim: besides the well-known bone-related vitamin D functions, it has immunomodulatory proprieties. Brain death (BD) causes a massive catecholamine release, leading to intense inflammatory activity. The aim of this study was to evaluate serum levels of vitamin D in brain-dead donors in comparison with critically ill control patients and to evaluate if there are correlations between vitamin D and cytokine levels. **Methods:** forty-one brain-dead donors and 32 critically ill control patients admitted to intensive care unit (ICU) were prospectively included in the study. Plasma TNF-α, IL-1β, IL-6, IL-8, IL-10, IFN-γ and serum vitamin D levels were compared between BD donors and controls using Student's *t* test. Spearman's test was used to assess the correlation between vitamin D and cytokine levels.**Results**: mean vitamin D levels were 16.39 ng/mL, with 52 patients (71.23%) been classified as vitamin D deficient (serum levels <20 ng/mL). Vitamin D levels were similar between brain-dead donors and control subjects (15.64 ± 6.95 ng/mL *vs* 17.40 ± 9.02

ng/mL; P =0.383). Moderate direct correlations were observed between vitamin D and IL-8, IL-10, and IFN-γ in BD patients (IL-8: r=0.5, P=0.049; IL-10 r=0.67, P=0.005; IFN-γ r=0.6, P=0.015). As expected, an inverse correlation between vitamin D and IL-6 (r=-0.36, P=0.044) was found in critically ill controls.**Conclusions:** vitamin D serum levels are similar in BD and in other critical illnesses. In brain-dead donors, vitamin D serum levels correlated with plasma IL-8, IL-10 and IFN-γ.

Introduction

Organ transplantation is the treatment of choice for several end-stage diseases affecting kidney, liver, heart, lung and pancreas (1). Currently, the main source of organs for transplantation is the brain-dead donor (2). Brain death (BD) is the complete and irreversible loss of brain function (3). It causes a massive catecholamine release, leading to an intense inflammatory activity that produces adverse effects on outcomes of transplanted organs (2, 4). BD has been associated with an increased risk of acute and chronic rejection (5) and with higher rates of primary graft dysfunction (6).

Vitamin D is a fat-soluble vitamin synthesized by the skin in response to sunlight exposure. Normal levels of 25-hydroxyvitamin D (25 (OH) D) relay on skin synthesis stimulated by ultraviolet radiation and/or adequate dietary intake (7-10). Its half-life is approximately two to three weeks (10). Serum levels of 25 (OH) D are constant and representative of vitamin D stocks. More recently, advances in molecular and genetic fields showed vitamin D to have a wider range of effects than previously thought. These so-called pleiotropic effects include changes in inflammatory profile, as well as cardioprotective and immunomodulatory effects (11-13). These non-skeletal actions may be related to the presence of vitamin D receptors in various organs and cell types, besides those classically related to calcium metabolism (14-15).

Vitamin D deficiency is associated with a variety of conditions, such as autoimmune diseases, cancer, glucose metabolism disorders, and mortality (16). In critically ill patients, vitamin D deficiency has been associated with higher infection rates, longer time on mechanical ventilation, longer length of hospital stay, and higher mortality, but the evidence is still conflicting (17-20). Importantly, it is unknown whether vitamin D deficiency at the time of intensive care unit (ICU) admission affects survival of critically ill patients or if it is just a disease severity marker (21). In addition,

there is some evidence that critical illness might affect vitamin D levels, leading to its decrease during ICU length of stay (20, 22).

Vitamin D levels decrease during acute diseases that are associated with increased inflammatory activity (23, 24). Besides, increased concentrations of inflammatory markers, such as tumor necrosis factor-α (TNF-α) or C-reactive protein, are inversely correlated with vitamin D levels, suggesting that inflammatory process is a factor involved in low levels of vitamin D (21, 25, 26). Furthermore, as vitamin D supplementation is associated with decreased inflammation in some autoimmune diseases (27), it is possible that a critically ill patient with higher vitamin D levels would be protected from the exaggerated inflammatory response observed in BD.

Based on the exposed evidence, we hypothesize that vitamin D serum levels are associated with cytokine levels in BD subjects. Therefore, the aim of this study was to determine vitamin D levels in brain-dead patients as compared to critically ill patients and its possible association with inflammatory activity, by means of TNF-α, interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10) and interferon-γ (IFN-γ) measurements.

Patients and Methods

Brain-dead patients and controls

The study protocol was approved by the ethics committee at Hospital de Clínicas de Porto Alegre. Informed consent was obtained from patients or their next of kin. Brain death was assessed independently by two physicians and was based on the following criteria: coma with complete unresponsiveness, absence of brain stem reflexes, apnea test, and confirmatory exam with absence of cerebral blood flow, according to Brazilian law (28). From June 2013 to June 2015, brain-dead patients older than 18 years admitted to ICU were prospectively included in the study after the first clinical exam consistent with brain death. Control subjects were defined as critically ill patients without a suspected diagnosis of brain death admitted to the same ICU of the cases. For each brain-dead patient, two control patients were included: the first septic patient to the right and the first non-septic patient to the left. Sepsis was defined as the presence of infection leading to a new organ dysfunction (29). Clinical and laboratory data were recorded for brain dead and control patients. Blood samples were collected once at study entrance. Twenty-five blood samples

from brain-dead patients from a previous study (30) stored at -80 °C freezer were also used for vitamin D quantifications.

Plasma TNF-α, IL-1β, IL-6, IL-8, IL-10 and IFN-γ quantifications

Blood was immediately centrifuged at 1260 g for 10 min at 4ºC and plasma was stored at -80ºC until analysis. All samples were analyzed at the same time after being defrosted at room temperature and centrifuged at 1000 g for 10 min. Plasma levels of TNF-α, IL-1β, IL-6, IL-8, IL-10 and INF-γ were assessed by magnetic bead assay using the Human Magnetic Custom Luminex® Kit (Invitrogen Life Technologies, Carlsbad, USA) and the Luminex® 200™ magnetic bead plate reader (Luminex, Austin, USA) following the manufacturer's recommendations. Standard curve was generated by serially diluting the reconstituted standard. Samples and standards were incubated with mixed beads overnight at room temperature on an orbital shaker. Beads were washed and then incubated with a detection antibody at room temperature for 1 h and with streptavidin for 30 min. Beads were then washed and resuspended and the plate was subsequently analyzed on the Luminex® 200™. Results are reported as a function of fluorescence intensity. Mean fluorescence intensity (MFI) takes into account the number of fluorescing pixels within the scanned area. Values of MFI under the detection limit were assumed to be equal to lower detected result. MFI was then transformed to pg/mL based on the standard curve. Samples were analyzed in duplicates.

Serum Vitamin D quantifications

Blood samples were protected from sunlight exposure, centrifuged, stored at -80ºC and then processed simultaneously. 25 [OH] D3 levels were measured by chemiluminescence method (Liaison; Diasorin, Stillmater, Minnesota; inter and intra-assay coefficient of variation, 10%) and reported in ng/mL.

Statistical Analysis

Variables with normal distribution are presented as mean ± SD. Variables with skewed distribution are presented as median and interquartile intervals and were log transformed for statistical analysis. Categorical variables are presented as percentages. Baseline characteristics and serum vitamin D levels were compared between BD subjects and controls using Student's *t* test and among BD subjects,

septic and non-septic controls by One-way ANOVA. Spearman's test was used to assess correlations between serum vitamin D levels, clinical, laboratory variables and cytokine plasma levels (TNF-α, IL-1β, IL-6, IL-8, IL-10, IFN-γ). Values were considered statistically significant if P <0.05. This study was powered to find a correlation of 0.6 between vitamin D serum levels and cytokines, considering an alfa-error of 5% and a beta-error of 20%. All statistical analyses were performed using SPSS 18.0 (Chicago, IL).

Results

The characteristics of 41 brain-dead patients and 32 critically ill control patients included in the study are summarized in Table1. Stroke was the leading cause of BD (53.5%), followed by anoxic encephalopathy (13.9%). Main sites of infection in septic patients were lung (40.0%) and abdomen (26.6%). Non-septic patients were admitted to the ICU due to pulmonary embolism (20%), hemorrhagic stroke (20%), post-cardiac arrest (13.3%), elective cardiac surgery (13.3%) or other causes (33.4%). The majority of the characteristics were similar between brain-dead donors and controls. As expected, BD patients were more frequently on vasopressor support, had lower body temperature and higher levels of plasma sodium (Table 1).

Brain death and vitamin D levels

Mean vitamin D levels were 16.39 ng/mL. Sixteen patients (21.9%) were vitamin D insufficient (serum levels 20-29.9 ng/mL) and 52 patients (71.23%) were vitamin D deficient (serum levels <20 ng/mL). Vitamin D mean levels were similar between brain-dead donors and control subjects (15.64 ± 6.95 *vs* 17.40 ± 9.02 ng/mL; P =0.383), as shown in Figure 1. When controls were divided into septic and non-septic individuals, mean levels were also similar (brain-dead: 15.64 ± 6.95 *vs.* septic: 16.47 ± 8.62 *vs.* non-septic: 18.22 ± 9.60 ng/mL, P = 0.551).

As expected, serum levels of vitamin D were inversely related to BMI and body weight (BMI: r=-0.25, P=0.035; body weight: r=-0.26, P=0.024) (Figure 2). However, vitamin D serum levels did not correlate with any other clinical or laboratory variables (Table 2).

Vitamin D levels and inflammatory activity

Table 3 shows the correlations between vitamin D levels and plasma cytokines. Notably, a moderate positive correlation was observed between vitamin D serum levels and IL-10 (r=0.67, P=0.005) in BD patients. Besides, a similar association was found for IL-8 (r=0.50, P=0.049) and IFN-γ (r=0.60, P=0.015) (Table 3). Conversely, an inverse correlation between vitamin D serum levels and IL-6 was detected in the control group (r=-0.36, P=0.044). We also performed a subgroup analysis with subjects with vitamin D levels below 12 ng/mL, as this was the cutoff value associated with higher mortality in ICU in a previous study (20). However, no correlations were found between vitamin D and cytokines in this analysis (Table 3).

Discussion

In this sample of ICU patients, vitamin D serum levels were similar in brain-dead and critically ill control patients. Notably, there were a moderate positive correlation between certain plasma cytokines (IL-10, IL-8 and IFN-γ) and serum vitamin D levels in brain-dead donors.

Previous studies from our group have consistently demonstrated that BD induces inflammation in experimental models of BD (31, 32). More importantly, we demonstrated the upregulation of IL-6 and TNF-α levels in plasma as well as *TNF-α* mRNA expression in human pancreatic tissue from brain-dead donors as compared to control patients undergoing pancreatectomy for malignant tumors (30, 33)). Recently, we establish that BD is associated with a higher systemic inflammation than that induced by critical illness and to a similar level to that observed in sepsis, as demonstrated by the upregulation of IL-6, IL-8, IL-10, INF-γ and TNF-α in brain-dead in comparison with non-septic critically ill control patients (Schwarz P, data in preparation).

Vitamin D deficiency is associated with increased inflammation and development of autoimmune disorders (15, 21, 25, 31). In this case, vitamin D deficiency would have a causative role in the development of systemic inflammation. Interestingly, a novel theory suggests the association between vitamin D and inflammation in the opposite direction (34). These authors argue that bacteria may infect immune cells, such as monocytes and macrophages, and induce the intracellular conversion of 25 (OH) D into 1,25 (OH) D by 1-α-hydroxylase (CYP27B1) activation. The induction of this enzyme is, in part, mediated by an increase in inflammatory cytokines. The increase in 1,25 (OH) D would decrease the

substrate (25 (OH) D), which explains how inflammation would cause lower 25 (OH) D serum levels (34). As BD induces a highly inflammatory state (6, 30), we were expecting vitamin D levels to be lower in these patients. By contrast, we found similar levels of vitamin D between brain-dead patients and other critically ill subjects, including septic and non-septic patients. Vitamin D deficiency is a common condition in ICU patients at admission (20, 35). In our sample, the majority of patients (71.23%) had levels of vitamin D considered as deficient, which could partially explain our negative results.

1,25 dihydroxyvitamin D regulates the immune function in several ways. Initially, it promotes innate immunity, by activating macrophages via toll-like receptors (TLR) (36). The activation of monocyte TLR-2 signals the transcriptional induction of vitamin D receptor (*VDR*) and 1-hydroxylase (*CYP27B1*) genes. Serum 25 (OH) D bounds to serum vitamin D binding protein and enters monocytes, where the conversion to 1,25-dihydroxyvitamin takes place. 1,25-dihydroxyvitamin, in turn, acts as a transcriptional factor, inducing expression of cathelicidin (*LL37*), an antimicrobial peptide (36). This is the reason vitamin D deficiency is associated with increased risk for respiratory infections, especially tuberculosis. Additionally, vitamin D suppresses adaptive immunity by inhibiting the maturation of antigen presenting cells (APC), reducing their capacity to present antigen to CD4 cells and further inhibiting their proliferation and differentiation (37). The activation of CD4 cells leads to production of several cytokines (38). Thus, vitamin D deficiency is associated with an increase in the inflammatory (IL-1, IL-2, IL-6, IL-8, TNF-α and INF-γ) and a decrease in the anti-inflammatory cytokines (IL-4, IL-10 and interleukin-13 (IL-13)), affecting the anti-inflammatory/pro-inflammatory ratio (39, 40). In fact, vitamin D boosts mucosal defenses and dampers excessive inflammation (41), indicating a role of vitamin D in host defenses, modulation of inflammation and development of autoimmune disorders (15, 21, 25, 31).

In our study, vitamin D directly correlates with IL-8, IL-10 and IFN-γ in BD patients (Table 3). The positive correlation between IL-10 and vitamin D was expected due to its anti-inflammatory role. However, the positive correlations with IL-8 and IFN-γ were unpredicted because of their pro-inflammatory profile. These novel findings reflect the high complexity actions of vitamin D in immune system balance. In order to understand our unexpected positive correlation between vitamin D and plasma levels of the inflammatory cytokines IL-8 and IFN-γ, we may speculate that

BD causes major changes in gene expression signature, possibly affecting the *VDR* gene. It is unknown if BD modifies the transcriptome in humans, but it may possibly be predicted as BD causes profound metabolic disturbances. In line with this, IL-8, a chemical signal that attracts neutrophils to the site of inflammation, is upregulated in patients with sepsis (42) and has been suggested as one of the best predictors of sepsis progression and mortality in patients with sepsis (43, 44). The relationship between polymorphism of *IL8* gene and probability of sepsis progression has previously been demonstrated (44, 45). Nevertheless, no studies evaluated the clinical relevance of certain cytokine or *VDR* genotypes in association with more susceptibility to BD-induced inflammation.

This was the first study to measure vitamin D serum levels in brain-dead patients, but we must address some limitations. First, we did not exclude patients in-hospital before ICU admission, but as vitamin D has a long half-life, the previous in-hospital period probably did not affect the results. Second, serum 25 (OH) D serum not always reflect the levels of the active form 1,25 dihydroxyvitamin D (34), and we did not measure this molecule, which could have been elevated even in the presence of low levels of 25 (OH) D. However, 25 (OH) D represents well the vitamin D stocks and is the usual measured metabolite. Furthermore, even the measurement of 1,25 dihydroxyvitamin D may be misleading, as intracellular conversion mediated by 1-α-hydroxylase occurs in the cytoplasm of immune cells (36). In addition, 85 % of vitamin D is transported in blood bounded to vitamin D binding protein and 15% bounded to albumin. Serum levels of these proteins are prone to variations related to the clinical status of patients and may decrease in liver diseases as well as poor nutrition, both common conditions in the ICU setting. Finally, the sample size was calculated to find a correlation of at least 0.6, thus we cannot exclude the absence of other correlations with smaller magnitude.

In summary, vitamin D serum levels were not different in brain-dead as compared to other critically ill patients. However, a moderate positive correlation was found between vitamin D serum levels and plasma levels of IL-8, IL-10 and IFN-γ. We cannot exclude that BD might be causing modifications in vitamin D enzymes metabolism as well as in *VDR* gene expression. Then, transcriptomic studies would help to clarify the relationship between vitamin D and inflammation occurring during BD.

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Author's contributions: G.C. designed the study, researched data, performed analyses, and drafted the manuscript. C.B.L. and T.H.R. designed the study, performed analyses, and revised the manuscript. P.S. researched data. M.C. and D.C. designed the study, contributed to the discussion and reviewed the last version of the manuscript. T.H.R is the guarantor of this work and, as such, has full access to all the data and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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REFERENCES

1. Rech TH, Rodrigues Filho, EM. Manuseio do Potencial Doador de Múltiplos Órgãos. Revista Brasileira de Terapia Intensiva. 2007;19(2):197-204.

2. Nagata H, Matsumoto S, Okitsu T, et al. Procurement of the human pancreas for pancreatic islet transplantation from marginal cadaver donors. Transplantation. 2006;82(3):327-31.

3. Wijdicks EFM. The landmark “Le Coma Dépassé”. In: Wijdicks EFM. Brain death. Philadelphia: Lippincott Williams & Wilkins; 2001.

4. Powner DJ, Hendrich A, Lagler RG, et al. Hormonal Changes in Brain Dead Patients. Crit Care Med. 1990;18(7):702-8.

5. Pratschke J, Wilhelm MJ, Laskowski I, et al. Influence of donor brain death on chronic rejection of renal transplants in rats. Journal of the American Society of Nephrology : JASN. 2001;12(11):2474-81.

6. Jassem W, Koo DD, Cerundolo L, Rela M, Heaton ND, Fuggle SV. Leukocyte infiltration and inflammatory antigen expression in cadaveric and living-donor livers before transplant. Transplantation. 2003; 75(12):2001-7.

7. Pramyothin P, Holick MF. Vitamin D supplementation: guidelines and evidence for subclinical deficiency.Curr Opin Gastroenterol. 2009;28:139-50.

8. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab. 2011;96:1911-1930.

9. Biesalski HK. Vitamin D recommendations: beyond deficiency. Ann NutrMetab. 2011;59:10-6.

10. Holick MF. Vitamin D: Photobiology, metabolism, and clinical applications. In: de Groot LC, ed. Endocrinology. 1995;990-1011.

11. Holick MF. Vitamin D deficiency. N Engl J Med. 2007; 357:266-281.

12. Souberbielle JC, Body JJ, Lappe JM, et al. Vitamin D and musculoskeletal health, cardiovascular disease, autoimmunity and cancer: recommendations for clinical practice. Autoimmun Rev. 2010;9:709-715.

13. Holick MF. Vitamin D deficiency in 2010: health benefits of vitamin D and & sunlight – a D-bate. Nat Rev Endocrinol. 2011;7:73-75.

14. Carthy EP, Yamashita W, Hsu A, Ooi BS. 1,25-Dihydroxyvitamin D3 and rat vascular smooth muscle cell growth. Hypertension. 1989;13:954–959.

15. Gysemans CA, Cardozo AK, Callewaert H, et al. 1,25-Dihydroxyvitamin D3 modulates expression of chemokines and cytokines in pancreatic islets: Implications for prevention of diabetes in non-obese diabetic mice. Endocrinology. 2005;146:1956–1964.

16. Chu MP, Alagiakrishnan K, Sadowski C. The cure of ageing: vitamin D--magic or myth? Postgrad Med J. 2010;86:608–16.

17. Matthews LR, Ahmed Y, Wilson KL, Griggs DD, Danner OK. Worsening severity of vitamin D deficiency is associated with increased length of stay, surgical intensive care unit cost, and mortality rate in surgical intensive care unit patients. Am J Surg. 2012;204:37-43.

18. Arnson Y, Gringauz I, Itzhaky D, Amital H. Vitamin D deficiency is associated with poor outcomes and increased mortality in severely ill patients. QJM. 2012;105:633-9.

19. Flynn L, Zimmerman LH, McNorton K, et al. Effects of vitamin D deficiency in critically ill surgical patients. Am J Surg. 2012;203:379-82.

20. Moraes RB, Friedman G, Wawrzeniak IC et al. Vitamin D deficiency is independently associated with mortality among critically ill patients. Clinics. 2015;70(5):326-332.

21. Autier P, Boniol M, Pizot C, Mullie P. Vitamin D status and ill health: a systematic review. Lancet Diabetes Endocrinol. 2014;2:76-89.

22. Higgins DM, Wischmeyer PE, Queensland KM, Sillau SH, Sufit AJ, Heyland DK. Relationship of vitamin D deficiency to clinical outcomes in critically ill patients. JPEN J Parenter Enteral Nutr. 2012;36:713-20.

23. Van den Berghe G, Van Roosbroeck D, Vanhove P, et al. Bone turnover in prolonged critical illness: effect of vitamin D. J Clin Endocrinol Metab. 2003;88: 4623-32.

24. Lee P, Eisman JA, Center JR. Vitamin D deficiency in critically ill patients. N Engl J Med. 2009;360:1912-14.

25. Bellia A, Garcovich C, D`Adamo M, et al. Serum 25-hidroxyvitamin D levels are inversely associated with systemic inflammation in severe obese subjects. Intern Emerg Med. 2013;8: 33-40.

26. Peterson CA, Heffernan ME. Serum tumor necrosis factor-alpha concentrations are negatively correlated with serum 25(OH)D concentrations in healthy women. J Inflamm (Lond). 2008; 24:5-10.

27. Bhargava P, Cassard S, Steele SU, et al. The Vitamin D to Ameliorate Multiple Sclerosis (VIDAMS) trial: Study design for a multicenter, randomizer, doble – blind controlled trial of vitamin D in multiple sclerosis. Contemporary Clinical Trials. 2014;39(2): 288-293.

28. Dispõe sobre a remocão de órgãos, tecidos e partes do corpo humano para fins de transplante e tratamento e dá outras providências, Lei No. 9.434, de 4 de fevereiro de 1997 (1997).

29. Singer Meryn, Deutschman CS, Seymour CW; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA. 2016;315(8):801-810.

30. Rech TH, Crispim D, Rheinheimer J, et al. Brain Death induced inflammatory activity in human pancreatite tissue: A case control study. Transplantation. 2014;97(2):212-219.

31. Carlessi R, Lemos NE, Dias AL, et al. Exendin-4 attenuates brain death-induced liver damage in the rat. Liver Transplant. 2015;21(11):1410-8.

32. Carlessi R, Dias AL, Bauer A, et al. Exenatide protects pancreatic islets against brain death-induced inflammation and viability loss. Endocrine Abstracts. 2014;35:341.

33. Brondani LA, Rech TH, Boelter G, et al. UCP2 expression is increased in pancreas from brain-dead donors and involved in cytokine-induced β cells apoptosis. Transplantation. 2017;101(3):59-67.

34. Mangin M, Sinha R, Fincher K. Inflammation and vitamin D: the infection connection. Imflamm. Res. 2014;63:803-819.

35. Lucidarme O, Messai E, Mazzoni T, et al. Incidence and risk factors of vitamin D deficiency in critically ill patients: results from a prospective observational study. Intensive Care Med. 2010; 36:1609-11.

36. Hewison M. Vitamin D and immune function: an overview. Proc Nutr Soc. 2012;71(1):50-61.

37. Hewison M. Vitamin D and the imune system: new perspectives on an old theme. Endocrinol Metab Clin North Am. 2010;39(2):365-379.

38. Zhu J, Yamane H, Paul WE. Differentiation of Effector CD4 T Cell Populations. Annu Rev Immunol. 2010;28:445-489.

39. Baeke F, Takiish T, Korf H, et al. Vitamin D: modulator of the immune system. Curr Opin Pharmacol. 2010;10:482-96.

40. Baeke F, Gysemans C, Korf H, et al. Vitamin D insufficiency : implications for the imune system. Pediatr Nephrol Hypertens. 2008;17:348-52.

41. Pfeffer PE, Hawrylowicz CM. Vitamin D and Lung disease. Thorax. 2012;67(11):1018-20.

42. Hu D, Wang H, Huang X et al. Investigation of associaation between IL-8serum levels and IL8 Polymorphisms in Chinese patients with sepsis. Gene. 2016;594(1):165-170.

43. Mera S, Tatulescu D, Cismaru C, et al. Multiplex cytokine profiling in patients with sepsis. 2011;119(2):155-163.

44. Wacharasint P, Nakada TA, Boyd JH, Russell JA, Walley KR. AA genotype of IL-8 -251A/T is associated with low PaO_2/ FIO_2 in critically ill patients and with increased IL-8 expression. Respirology. 2012;17(8): 1253-1260.

45. Yousef AAA, Suliman GA, Mabrouk MM. The value of admission serum IL-8 monitoring and the correlation with IL-8 (-251A/T) polymorphism in critically ill patients. ISRN Inflammation. 2014;494985.

Table 1. Baseline characteristics of brain-dead patients and controls.

	Brain-dead (n=41)	Controls (n= 32)	P
Age (yr)	51 ± 12	50 ± 19	0.847
Male, n (%)	21 (28.7)	19 (26.0)	0.487
BMI (Kg/m^2)	25 ± 3	28 ± 8	0.720
Sepsis, n (%)	4 (8.3)	16 (33.3)	0.098
Time from ICU admission (days)	5.1 ± 6	6.4 ± 7	0.555
Ventilation support (days)	5.1 ± 6	4.6 ± 6	0.809
Vasopressor support, n (%)	34 (46.5)	13 (17.8)	<0.001
Episode of cardiac arrest, n (%)	9 (13.8)	3 (4.6)	0.063
Use of steroids, n (%)	17 (26.1)	12 (18.4)	0.321
Hemoglobin (g/dL)	10.01 ± 2.28	8.99 ± 1.91	0.117
White blood count (per mm^3 x 1000)	12,58	13,88	0.751
Creatinine (mg/dL)	1.8 ± 2.2	1.38 ± 1.06	0.333
Plasma sodium (mEq/L)	155 ± 8	141 ± 5	<0.001

BMI: body mass index.

Table 2. Correlation between characteristics of brain-dead patients and serum vitamin D levels

	Vitamin D	
	R	P
BMI	-0.25	0.035
Sepsis, (%)	0.08	0.564
Time from ICU admission (days)	-0.01	0.957
Ventilation support (days)	0.01	0.941
Vasopressor support (days)	0.04	0.848
Use of steroids (%)	0.27	0.290
Hemoglobin (g/dL)	0.39	0.794
Creatinine (mg/dL)	0.15	0.310
Plasma sodium (mEq/L)	0.03	0.825

BMI: body mass index. Spearman's test was used for correlation. Correlation is significant at $P< 0.05$.

Table 3.Correlation between plasma cytokines and serum vitamin D levels

	Vitamin D (all patients)	Vitamin D (BD patients)	Vitamin D (controls)	Vitamin D (levels <12 ng/mL)
TNF-α	r 0.14 P=0.328	r 0.09 P=0.636	r 0.09 P=0.623	r 0.19 P=0.455
IL-6	r -0.15 P=0.319	r 0.33 P=0.063	r -0.36 P = 0.044	r 0.29 P=0.240
Il-1β	r 0.12 P=0.405	r 0.13 P=0.494	r -0.02 P=0.914	r 0.13 P=0.605
IL-8	r 0.033 P=0.826	r 0.50 P=0.049	r -0.15 P=0.398	r –0.27 P= 0.358
IL-10	r 0.00 P=0.999	r 0.67 P=0.005	r -0.26 P=0.155	r -0.22 P=0.316
IFN-γ	r 0.16 P=0.269	r 0.60 P=0.015	r -0.01 P=0.971	r -0.06 P=0.831

Spearman's test was used for correlation. Correlation is significant at P <0.05.

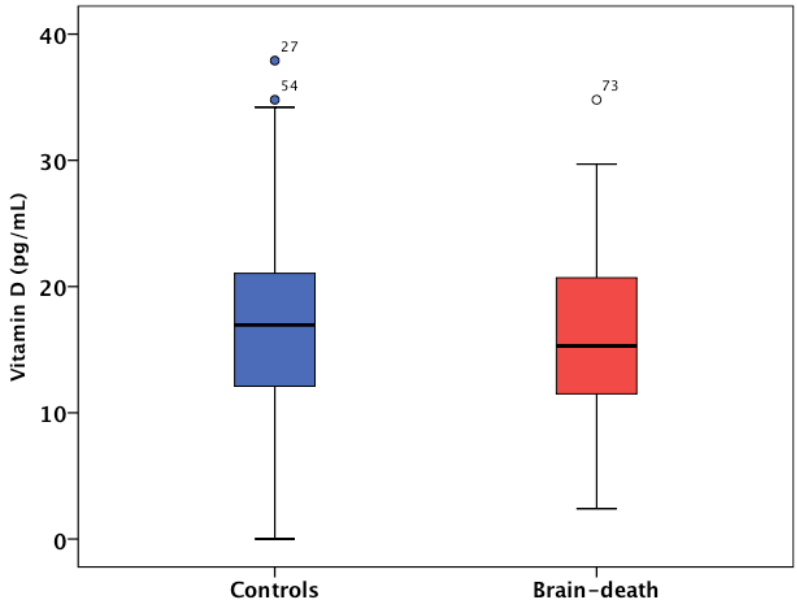

Figure 1 - Vitamin D serum levels are similar between brain-dead donors and critically ill control subjects.

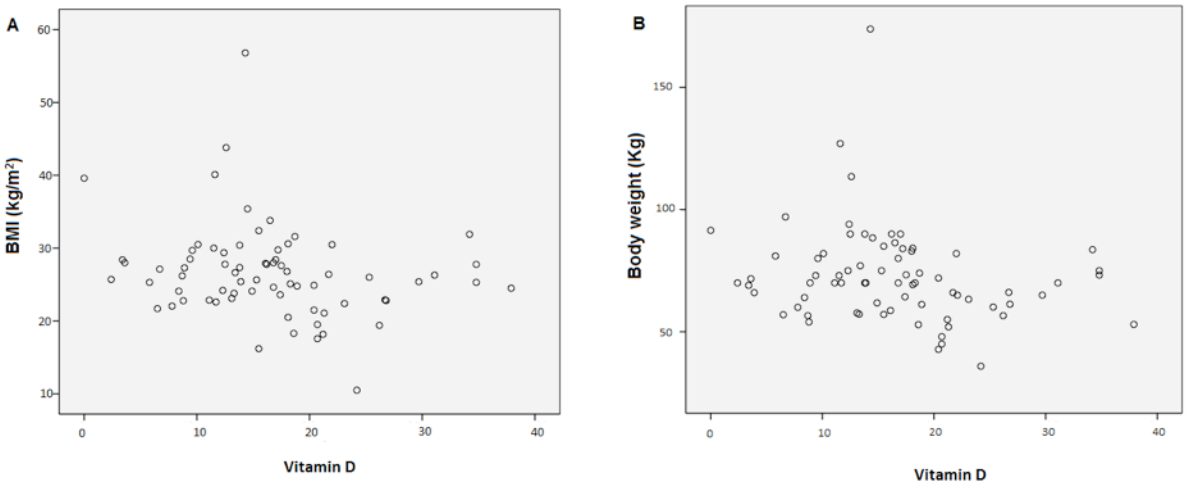

Figure 2 - Vitamin D serum levels are inversely related to BMI and body weight. A. BMI. B. Body weight. BMI= body mass index.

CAPÍTULO 3

CONSIDERAÇÕES FINAIS E PERSPECTIVAS FUTURAS

Existe no mundo inteiro um desequilíbrio entre a demanda e a oferta de órgãos para transplante.[1] No Brasil, que é o maior sistema público de transplantes do mundo, com cerca de 20.000 transplantes realizados anualmente,[2] o problema é crônico e crescente (Figura 1).

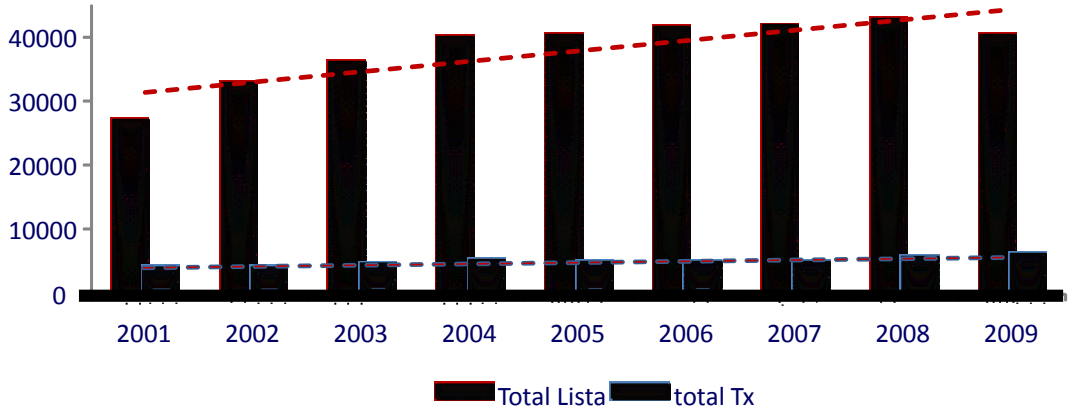

Figura 2 - Transplantes de órgãos sólidos no Brasil: lista de espera (em vermelho) *versus* número de transplantes realizados (em azul) entre 2001 e 2009. Dados do Registro Brasileiro de Transplantes da Associação Brasileira de Transplante de Órgãos.

Após a recusa familiar em doar, a perda de doadores por parada cardíaca é a principal causa de perda de doadores.[3] Desta forma, existe um espaço para melhorar os cuidados com o potencial doador de órgãos. Estudos observacionais demonstraram que o uso de *check lists* para guiar a manutenção dos doadores é capaz de reduzir a perda de doadores por parada cardíaca.[3,4] O Ministério da Saúde do Brasil tem dado grande relevância a essa questão, o que culminou no financiamento de um projeto denominado DONORS, que se constitui em estratégias para otimizar a doação de órgãos no Brasil. Este estudo é um ensaio clínico randomizado por clusters, onde 70 unidades de terapia intensiva (UTI) em todo o Brasil serão alocadas para um grupo intervenção (implantação de protocolo clínico baseado nas diretrizes brasileiras de manutenção do potencial doador de órgãos) ou

para um grupo controle (manutenção da rotina usual de cuidados do centro). O Hospital de Clínicas de Porto Alegre (HCPA) irá participar do estudo, o que abre a possibilidade de propor sub-estudos ao projeto principal, uma vez que o nosso grupo de pesquisa tem-se dedicado ao estudo da morte encefálica (ME), principalmente nas questões relacionadas à inflamação induzida pela ME.[5,7]

No presente estudo não identificamos diferenças nos níveis séricos de vitamina D nos pacientes em ME quando comparados a doentes críticos sem ME, mas encontramos uma correlação direta entre vitamina D e a citocina anti-inflamatória interleucina-10 (IL-10). Ao contrário do esperado, a interleucina-8 (IL-8) e o interferon-y (INF-γ), ambas citocinas inflamatórias, foram diretamente correlacionados com os níveis séricos de vitamina D. Isso nos levou a considerar quais fatores poderiam explicar esta associação inesperada. Como a ação da vitamina D depende de outras fatores além do nível sérico de vitamina D (25 (OH) D), tais como proteínas carreadores plasmáticas, atividade da 1-alfa-hidroxilase renal e em outros tecidos, além da expressão de receptores da vitamina D, a questão de pesquisa que se apresenta a seguir é se a ME altera cada um destes fatores possivelmente envolvidos no metabolismo da vitamina D. Tendo como base os achados acima descritos, pretendemos submeter ao centro coordenador do projeto DONORS alguns sub-estudos. O principal deles é um estudo que propõe a realização de sequenciamento de RNA (RNA seq) de cinco doadores em ME e cinco controles com lesão neurológica grave sem ME, com o objetivo de definir a assinatura inflamatória da ME e de analisar quais vias de sinalização intracelulares são modificadas pela ME. Outro estudo que será proposto terá como objetivo a determinação da presença de polimorfismos nos genes das citocinas e seus efeitos na inflamação induzida pela ME, uma vez que nada se sabe sobre polimorfismos de citocinas e ME.

Além disso, outro projeto com o objetivo de entender melhor o papel da vitamina D nos mecanismos da inflamação da ME deve incluir dosagens de citocinas, vitamina D e sua proteína carreadora, albumina e 1,25-hidroxivitamina D em uma amostra maior de pacientes do que a do estudo atual, oportunidade única oferecida pelo desenvolvimento do projeto DONORS.

Por fim, em relação ao tratamento da inflamação induzida pela ME, a exenatida, um análogo sintético do *glucagon like peptide-1* (GLP-1) usado no tratamento do diabetes tipo 2, tem propriedades anti-inflamatórias, citoprotetoras e

antiapoptóticas em ilhotas pancreáticas isoladas de humanos[8] e de ratos em ME,[6,7,9] como demonstrado nesse estudo do nosso grupo. Desta forma, pretendemos testar por meio de um ensaio clínico randomizado em doadores em ME os efeitos da exenatida em atenuar a inflamação induzida pela ME em biópsias de órgãos captados para transplante e a sua capacidade de reduzir a ocorrência de disfunção primária desses enxertos.

REFERÊNCIAS

1. Sheehy E, Conrad SL, Brigham LE, et al. Estimating the number of potencial organ donos in the United States. N Engl J Med.2003;349: 667-74.

2. Associação Brasileira de Transplantes de Órgãos (ABTO). Dimensionamento dos transplantes no Brasil e em cada estado (2006-2013). 2013;19(4). Disponível em: http://www.abto.org.br/abtov03

3. Westphal GA, Coll E, de Souza RL, et al. Positive impact f a clinical goal-directed protocolo on reducing cardiac arrests during potencial brain- dead donor maintenance. Crit Care 2016;20:323.

4. Franklin GA, Santos AP, Smith JW. Optimization of donor management goals yields increased organ use. Am Surg. 2010;76(6):587–94.

5. Rech TH, Crispim D, Rheinheimer J, et al. Brain Death induced inflammatory activity in human pancreatite tissue: A case control study. Transplantation. 2014;97(2):212-219.

6. Carlessi R, Lemos NE, Dias AL, et al. Exendin-4 attenuates brain death-induced liver damage in the rat. Liver Transplant. 2015;21(11): 1410-8.

7. Carlessi R, Dias AL, Bauer A, et al. Exenatide protects pancreatic islets against brain death-induced inflammation and viability loss. Endocrine Abstracts. 2014;35:341.

8. Cechin SR, Perez-Alvarez I, Fenjves E, et al. Anti-inflammatory properties of exanatide in human pancreatic islets. Cell transplant 2012;21:633-48.

9. Hering BJ, Kandaswamy R, Ansite JD, et al. Single-donor, marginal-dose, islet transplantation in patients with type 1 diabetes. JAMA 2015;293:830-5.