



# Laboratorial evaluation of potential donors of organs and tissues for transplantation

Avaliação laboratorial de potenciais doadores de órgãos e tecidos para transplantes

Evaluación de laboratorio de potenciales donantes de órganos y tejidos para trasplante

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The objective of this study was to describe the laboratorial complementary evaluation in potential donors of organs and tissues for transplantation. It is a descriptive, quantitative study made in six hospitals in Natal/ Rio Grande do Norte, Brazil, between August/2010 to February/2011. The sample consisted of 65 potential donors and a checklist type instrument was used. Information was collected and analyzed using descriptive statistics. From a total number of donors, 89.2% had blood typing, 80.0% hematological tests and verification of the electrolytes. As for the functions, 70.8% had tests for verification of pulmonary function and 80.0% for renal function. From the alterations detected, 69.2% presented hyperoxia, 66.2% leukocytosis, 47.7% hypernatremia, 43.1% increase in the creatine kinase, 10.0% with positive serology. Relevant tests were not made. It is essential to assess potential donors to detect and treat alterations, ensuring the quality of the organs and the quality of the transplantation.

**Descriptors:** Nursing; Tissue and Organ Procurement; Tissue Donors; Transplants; Brain Death.

Objetivou-se descrever a avaliação laboratorial e complementar em Potenciais Doadores de órgãos e tecidos para transplantes. Estudo descritivo, quantitativo, realizado em seis hospitais de Natal/Rio Grande do Norte, Brasil, entre agosto/2010 e fevereiro/2011. A amostra constituiu-se de 65 potenciais doadores e como instrumento utilizou-se uma lista de verificação. As informações foram coletadas e analisadas por estatística descritiva. Do total, 89,2% realizaram a tipagem sanguínea, 80,0% testes hematológicos e verificação dos eletrólitos, cada. Quanto às funções, 70,8% realizaram exames para a verificação da função pulmonar, 80,0% para a função renal. Das alterações detectadas, 69,2% apresentaram hiperóxia, 66,2% leucocitose, 47,7% hipernatremia, 43,1% elevação na creatina fosfoquinase, 10,0% com sorologia positiva. Exames importantes não foram realizados. É fundamental avaliar potenciais doadores para detectar e tratar alterações, garantindo a qualidade dos órgãos e realização do transplante.

**Descritores:** Enfermagem; Obtenção de Tecidos e Órgãos; Doadores de Tecidos; Transplantes; Morte Encefálica.

El objetivo fue describir la evaluación de laboratorio y complementaria en potenciales donantes de órganos y tejidos para trasplantes. Estudio descriptivo, cuantitativo, realizado en seis hospitales de Natal/Rio Grande do Norte, Brasil, entre agosto/2010 y febrero/2011. La muestra compuesta por 65 potenciales donantes y se utilizó como instrumento una lista de verificación. Recolectaron los datos, analizados por estadística descriptiva. Del total, 89,2% realizaron el tiraje de sangre, 80,0% análisis hematológicos y verificación de los electrolitos. Cuanto a las funciones, 70,8% fueron sometidos a exámenes de la función pulmonar y 80,0% de la función renal. De los resultados, 69,2% tenían hiperoxia, 66,2% leucocitosis, 47,7% hipernatremia, 43,1% aumento de la creatina quinasa, 10,0% serología positiva. No se realizaron pruebas pertinentes. Es esencial evaluar los posibles donantes para detectar y tratar los cambios, lo que garantiza la calidad de los órganos y el trasplante.

**Descritores:** Enfermería; Obtención de Tejidos y Órgãos; Donantes de Tejidos; Trasplantes; Muerte Encefálica.

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## Introduction

As time went by and with the technological advance in vital support, in the studies and in the techniques of resuscitating, the brain activity became the item which defines and distinguishes life and death of a person. Death became associated to neurological parameters and recognized as brain death<sup>(1)</sup>.

The Federal Council of Medicine of Brazil, in 1997, defined brain death through the resolution 1.480/97, as being 'the total and irreversible stop of the brain functions, from a known cause and recognized in an unquestionable way' after clinical and complementary exams, constituting death for social, legal and clinical effects<sup>(2)</sup>.

The confirmation of the medical diagnosis of brain death is made through the medical history and findings in the physical and complementary exams, which are mandatory by the Brazilian law<sup>(1)</sup>. Once the cause of the coma has been established, the following items must be detected in the patient: absence of the electrical or metabolic activity or brain blood perfusion, perceptible coma, apnea, absence of motor supra-spinal activity<sup>(2)</sup>.

The beginning of the clinical exam must occur in a hemodynamically stable person, and the exam must be repeated at least in two occasions, preferably by two doctors who were not participants in the teams of transplantation and removal, complying with the guidelines of the Federal Council of Medicine. The interval of time between the exams is specific for each age range, and it is six hours between them for patients older than two years of age and a complementary exam is optional<sup>(1,3)</sup>.

Regarding the donation of organs, the demand is still much higher than the supply, which makes the need to increase the number of donations constant. The objective of the agencies responsible for the capture of organs is to make the figures of demand and supply closer; this should also be objective of the professionals involved in the process of donation, especially in the care of the potential donor. Potential

donor of organs and tissues for transplantation is anyone who presents perceptible and reactive coma and there must not be any possibility of infectious disease which makes the donation unfeasible<sup>(4)</sup>.

The treatment provided for the potential donor is directly linked to the effectiveness and success of the transplantation<sup>(5)</sup>. The nurses have a fundamental role in this process, once they render direct services to the potential donors, working in the maintenance of the clinical and laboratorial parameters until the effectiveness of the transplantation. For the maintenance of the potential donor and prevention of organic malfunctions, it is necessary to have a profound knowledge of the physiopathological consequences of the brain death, that is why the growing interest in the care with potential donors<sup>(6)</sup>.

The brain death affects the homeostasis of several organs and systems, caused by irreversible hypothalamic lesion. These alterations can be reverted if there is correct care of the potential donor in order to minimize the adverse effects<sup>(7)</sup>. The objective is to reduce the progressive somatic deterioration of the body and keep or improve the function of the specific organ for transplantation. The perfusion of the organs must be kept to preserve the stability, quality and feasibility of the organ<sup>(6)</sup>.

An adequate laboratorial evaluation of the potential donor is essential to obtain the organs and tissues of quality, in order to avoid transmission of infectious diseases or neoplasias<sup>(8)</sup>. The physiological and biochemical alterations of the cells occur in all the organic systems and are able to affect the function of the potentially available organs for transplantation<sup>(1,9)</sup>.

The continuous and strict control and the register of the parameters are fundamental to keep the organic functions closer to normal<sup>(10)</sup>. Biochemical alterations after brain death can interfere in the quality of the graft transplanted. Among them there are the following disorders: endocrinous, metabolic, in the hydro-electrolyte balance, as respiratory alkalosis, hypokalemia, hyperkalemia, hypernatremia, hypophosphatemia and metabolic acidosis<sup>(9-12)</sup>.

Considering that the number of patients in waiting lists for transplantation is growing, while the number of donors is still insufficient to attend the demand, it is extremely important to know how the maintenance and the laboratorial evaluation of the potential donor is made<sup>(1)</sup>.

The Health Department through the Department of Attention to Health is responsible for the program of donation, capture and transportation of organs and tissues in order to reduce the waiting time in lines, optimizing the use of organs and tissues. However, despite the scientific, technological, organizational and administrative advance that contributes to the significant increase of the number of transplantations, it is still insufficient due to the accumulated search of organs, not only in Brazil, but all over the world<sup>(1)</sup>.

So, studying the process of donation of organs and tissues for transplantation, more specifically how the maintenance of the potential donor and the laboratorial and complementary evaluation is made concerning those subjects, all of them are necessary to detect frailties and faults which might interfere in the donation of organs and tissues for transplantation.

Concerned with the maintenance of the potential donor and consequently with the low rate of effectiveness of the transplantation, the following question is made: How is the laboratorial and complementary evaluation is carried out in potential donor for organs and tissues of transportation?

So, the objective of this study was to describe laboratorial and complementary evaluation carried out in potential donors of organs and tissues of transportation.

## Method

It is a descriptive study, with prospective and quantitative data, made in six hospital units in Natal/Rio Grande do Norte, Brazil, accredited by the National System of Transplantation for the removal and transplantation of organs and tissues, as well as in the Center of Notification, Capture and Donation

of Organs for Transplantation and Organization of Search for Organs in the state of Rio Grande do Norte, from August 2010 to February 2011.

The population of this study was made up by subjects in perceptive and reactive coma without any possibility of infectious disease. The sample was made up by 65 potential donors assisted in the six hospital units of Natal.

The criteria of inclusion in the sample were: present score three in the Glasgow Coma Scale; to have the cause of the coma determined through brain computerized tomography; have personal and family identification that can be responsible for the authorization or not, of the donation. The criteria of exclusion were: evidence of transmissible diseases, neoplasia, use of injected drugs which would make the donation unfeasible; improvement of the neurological status before the opening of the protocol of brain death.

The instrument of data collection used was elaborated specifically for this study, based on the fundamentals preconized by the scientific literature on the subject<sup>(1-2,4,13-15)</sup>. It is a structured script of non participant observation, like a list of verification made up by the following parts: data of personal identification, data on the evaluation, such as the activities developed by the health professionals with the potential donor and laboratorial alteration in the same. At this moment, the data regarding the identification and notification of the potential donor were collected, as well as the results of the clinical and laboratorial exams used for the evaluation of this patient; the procedures for the diagnosis of brain death and the care involved by the health professionals regarding the maintenance and feasibility of the organs and tissues of the potential donor.

For the data collection it was necessary to have a team made up by researchers and five nursing graduation students of the Universidade Federal do Rio Grande do Norte linked to the group of research Incubadora de Procedimentos de Enfermagem (Incubator of Procedures of Nursing) that works

in three shifts, with shifts of six and twelve hours. For the processual collection of data, an extension course of 40 hours was given, in which the nursing students learned, with theoretical and practical classes, the structure of the hospital units which were able to receive the potential donors and the stages of the process of donation of organs and tissues for transportation. From those 40 hours, 12 were theoretical, taught in the department of nursing of the Universidade Federal do Rio Grande do Norte and 28 were practices of observation in hospital units, under the supervision of a doctor's degree student. Once this stage was finished and the project was approved, the team started the collection of data, daily. The use of non participant systematic observation technique was used, besides the acquisition of institutional document, such as: register books and medical records.

For this study, the following exams and their respective values were considered, according to Figure 1<sup>(1-2,4,13-15)</sup>.

The data collected were organized in an electronic data bank, using a Microsoft Excel 2007 program sheet, which, after the correction and verification of typing mistakes through the validation by double typing, were exported to a statistic program. After that, the data were analyzed through descriptive statistics and were presented in charts.

After the approval of the project of research by the Committee of Ethics in Research of the University Hospital Onofre Lopes no. 414/10 and Certificate of Presentation for Ethical Appreciation no. 007.0.294.000-10, the information were collected and the consent of the spouse or a relative up to the second degree of the potential donor, to participate in the study, with the signing of the Informed Consent Form.

Laboratorial evaluation	Exams	Considered values
Daily	Hematological	Hemoglobin >10 g/dl; Hematocrit >30%; Leucogram: between 3,500 to 11,000 mm <sup>3</sup> ; Platelet: between 140,000 to 450,000 per microlite (uL)
	Electrolyte	Sodium: 135 to 145 mEq/L; Potassium: 3.5 to 5.5 mEq/L
	Pulmonary Function	Hydrogen potential: 7.35 to 7.45; Partial oxygen pressure: 80 to 100 mmHg; Partial carbonic gas pressure: 35 to 45 mmHg; <b>Oxygen saturation: 95 to 100%</b>
	Cardiac Function	Creatine kinase: man: 24-190 U/L and woman: 24-166 U/L; Creatine kinase of the Cardiac Muscle: > 25UI.
	Renal Function	Urea: 10 a 50 mg/dl; Creatinine: 0,4 to 1,4 mg/dl
	Hepatic function	Aspartate aminotransaminase: 7 to 40; Alanine aminotransaminase: 10 to 40; Gamma-Glutamyl Transferase: man 9-50 and woman: 8-40; Total Bilirubin: 0.1 to 1.0
	Pancreatic function	Blood glucose: 100-200 mg/dl; Amylase: 60-160.
Once	Blood typing	ABO classification and Rh factor
	Serology	Positive for human immunodeficiency virus, toxoplasmosis, syphilis, Chagas disease, hepatitis B and C, Cytomegalovirus, Human T- lymphotropic

**Figure 1** - Fundaments preconized by the scientific literature on the laboratorial and complementary evaluation of the potential donor of organs

## Results

Of the 65 potential donors, 50.8% were men and 53.8% were up to 45 years old. The most frequent diagnosis, among the participants were cerebral vascular accident (50.8%) and traumatic brain injury (44.6)%.

As to the laboratorial evaluation of the potential donors, there was a predominance of blood typing exam (89.2%), hematology (80.0%), electrolytes (80.0%) and renal function (80.0%), according to Table 1.

**Table 1** - Laboratorial evaluation of the potential donors of organs and tissues for transplantation

Laboratorial evaluation	Yes	No
	n (%)	n (%)
Blood typing	58 (89.2)	7 (10.8)
Hematology	52 (80.0)	13 (20.0)
Electrolytes	52 (80.0)	13 (20.0)
Renal function	52 (80.0)	13 (20.0)
Pulmonary function	46 (70.8)	19 (29.2)
Pancreatic function	24 (70.8)	41 (63.1)
Serology	20 (30.8)	45 (69.2)
Cardiac function	16 (24.6)	49 (75.4)
Hepatic function	11 (16.9)	54 (83.1)
Infection control	1 (1.50)	64 (98.5)

The serological tests (69.2%) and the cardiac (75.4%), hepatic (83.1%) and pancreatic functions (63.1%), were not made in a good parcel of the potential donors. As to the control of infections, the predominance of absence of exams in the potential donors was observed (98.5%).

Table 2 presents the laboratorial alterations of the potential donors.

**Table 2** - Laboratorial alterations present in potential donors of organs and tissues for transplantation

Laboratorial evaluation	n (%)
Hematology	
Leukocytosis	43 (66.2)
Anemia	25 (38.5)
Plateletpenia	20 (30.8)
Electrolytes	
Hypernatremia	31 (47.7)
Hypokalemia	30 (46.2)
Hypernatremia	15 (23.1)
Hyperkalemia	5 (7.7)
Blood gas analysis	
Acidosis	24 (36.9)
Hyperoxia	45 (69.2)
Hypocapnia	42 (64.6)
Alkalosis	21 (32.3)
Hypercapnia	9 (13.8)
Hypoxia	7 (10.8)
Cardiac enzymes	
Creatine kinase	28 (43.1)
Creatine kinase -MB	27 (41.5)
Renal evaluation	
> Urea	29 (44.6)
> Creatinine	29 (44.6)
Hepatic function	
Glutamic oxaloacetic transaminase	39 (60.0)
Glutamic- peruvic transaminase	24 (36.9)
Gamma-Glutamyl Transferase	16 (24.6)
Total Bilirubin	8 (12.3)
Direct Bilirubin	4 (6.2)
Pancreatic function	
Hyperglycemia	17 (26.2)
Hypoglycemia	12 (18.5)
< Amylase	17 (26.2)
> Amylase	10 (15.4)
Serology	
Positive	2 (10.0)

It was observed that 66.2% of the potential donors presented leukocytosis, 47.7% hypernatremia and 69.2% hyperoxia. As to the cardiac function, alterations were observed in Creatine Kinase (43.1%) and in the Creatine kinase -MB in 41.5%. In the renal function alterations in the markers of urea and creatine were found, with 44.6%, each. Concerning the hepatic function, 60.0% presented alterations in the Glutamic-oxaloacetic transaminase and 36.9% in the Glutamic-peruvic transaminase. In the pancreatic function, the potential donors presented hyperglycemia (26.2%) and decreasing in the rate of amylase enzyme (26.2%). As to serology, only 10.0% presented positive serology.

## Discussion

The socio-demographic and clinical profile of the potential donors is similar to other studies made in Brazil, in which there is a predominance of the male sex, at economically active age, single, widower or divorced, with low level schooling and having as the cause of brain death the cerebral vascular accident, followed by traumatic brain injury<sup>(15-18)</sup>.

The knowledge of the physiological alterations resulting from the inflammatory picture of the brain death by the nurse who is working in those sectors which receive these patients is fundamentally important so that, together with the medical team, they may lead the adequate therapeutical measures to the potential donor. When the maintenance of the patient under suspicion of brain death is inadequately made, it is observed that the complication throughout the process of clinical evaluation can reach 60% of the cases. In the past, with the adequate preparation for the clinical picture, this rate could be reduced for 7%<sup>(1,8)</sup>.

So, an adequate laboratorial evaluation of the potential donor of organs and tissues increases the probability to obtain a quality graft to be transplanted. It is important to emphasize that the hemodynamic instability, hematological disorders, hypothermia,

hydro electrolytes abnormalities, hormonal disorders and tissue hypo perfusion, are complications which interfere in the preservation of the organs and tissues. In this scope, the alterations must be early recognized, through laboratorial exams, functional tests and serology in order to detect the early alterations in the potential donor<sup>(8-9)</sup>.

In cases of donations of organs, the laboratorial exams are mandatory and these exams must be regularly made according to the protocols established, in order to identify hemodynamic, hematological, hydro electrolytes alterations, alteration in the serial concentrations, clot disorder and blood transmissible diseases, tissues or organs which can make the transplantation unfeasible.

Bearers of renal, cardiac, pulmonary, pancreatic and medullary insufficiency, of infect-contagious diseases such as the virus of human immunodeficiency I and II, of the markers for hepatitis B and C, Chagas disease and bearers of malign neoplasias are contraindicated for the process of donation of organs and tissues<sup>(8,11,19)</sup>. According to the literature, the laboratorial exams must include blood typing, serological, hematological, electrolytes exams, renal, hepatic, cardiac, pulmonary and pancreatic functions, blood culture and urine exam<sup>(8,19)</sup>.

In this study, the laboratorial exams made in the population researched were the blood typing, laboratorial hematological evaluation, electrolytes, pulmonary and renal functions. But, important exams such as the serological test and the pancreatic, hepatic and cardiac functions were not made in a good number of the potential donors, showing a fault in the process donation/transplantation of organs and tissues which directly influence in the number of potential donors, and, consequently, in the number of transplants.

The follow-up of these parameters aims at detecting and consequently treating alterations resulting from the brain death, at guaranteeing good functionality to the cardiac muscle and hemodynamic instability and decrease the loss of donors by a cardiovascular collapse and increase the post-

transplantation survival<sup>(4)</sup>. It is important to highlight that the collection of material for laboratorial exams must be made regularly according to the protocols established by the institutions<sup>(14)</sup>.

It was noticed that there was not a strict control of the infections, despite the fact that they are not considered a fact which could contraindicate the transplant. However, all the potential donors must be investigated as to the presence of infectious agents and anti-biotal therapy must be indicated in cases with suspicion or evidence of the infection<sup>(17)</sup>. The nurse and his team must take the universal precautions to prevent the infectious complications<sup>(1)</sup>.

Regarding the laboratorial alteration in the potential donors concerning hematology, 66.2% presented leukocytosis, however, because brain death is an inflammatory condition, the activation of inflammatory mediators as a complement, thromboxanes and leukocyte factors, have a central role in the events which permeate the brain death, so, leukocytosis isolated does not contraindicate donation<sup>(14)</sup>.

The plateletpenia was present in 30.8% of the potential donors, which highlights that the nursing team must be alert to the signs of clotting disorders, as gingivorrhagias, haematuria, or persistent bleeding in areas of vascular puncture<sup>(1)</sup>.

Regarding the electrolytes, 47.7% presented hypernatremia, an alteration which rises as a consequence of the diabetes insipidus, caused by the process of necrosis of the neuro-pituitary gland and depletion of the anti- diuretic hormone, and can be a predictor of the primary non-functioning of the hepatic graft<sup>(14,20-21)</sup>. Besides that, levels of sodium above 155mmol/L are intimately related to hepatic disorders and loss of the graft in the receptor<sup>(22)</sup>. The control of the hydro-electrolytes disorders must be made through the serial dosage of the electrolytes, and it is up to the nurse to detect any alteration which might happen in the sense with the potential donor<sup>(1)</sup>.

Concerning the rates of respiratory function, 69.2% presented hyperoxia, cause by oxidative

stress of the clinical picture of brain death. Abnormal values of the partial pressure of oxygen promote the liberation of the factor of tumoral alpha necrosis and interleukin 1-beta, which are inflammatory cytokines which permeate the pulmonary lesions, and they may make the graft unfeasible for donation. The blood gas analysis provides a reading of the partial pressures of oxygen and carbonic gas to the health professionals and are indispensable in the maintenance of the ventilator support of the donors<sup>(21,23)</sup>. It is essential that the nurse professionals who are involved in the maintenance of the potential donor have knowledge of the acid-basic balance and the respiratory physiology, in order to provide adequate assistance to the patient and avoid further complications<sup>(1)</sup>.

Concerning the cardiac alterations, some alterations of the Creatine kinase were identified in 43.1% of the potential donors and of the Creatine kinase-MB in 41.5%. A study made in 2011 emphasizes that the increase of the cardiac enzymes is common in deceased potential donor. Although it is related to the highest gravity of myocardial malfunction and failure of the transplant, the alterations in the cardiac enzymes do not necessarily indicate the presence of coronary disease, and so, they do not contraindicate the transplantation<sup>(21)</sup>.

In the evaluation of the renal function, 44.6% of the potential donors presented alterations in the markers of measure of urea and creatinine, each. In deceased donors in a critical situation it is common to occur rhabdomyolysis, which causes the sudden elevation of the plasmatic creatinine used as indicator of acute renal insufficiency, together with alterations in the volume of diuresis. However, it is considered a strong recommendation not to contraindicate the feasibility of the kidneys for transplantation based only in the alteration of the creatinine<sup>(21)</sup>.

Regarding the hepatic function 60.0% presented alteration in the Glutamic-oxaloacetic Transaminase and 36.9% Glutamic-peruvic Transaminase. The elevation of the transaminases and of the bilirubin can be an indicative of hepatic ischemia motivated by

the hypo perfusion or subclinical viral hepatitis. This fact does not make the transplantation unfeasible, but, it should warn the transplanting team to monitor the arrival of complications or viral hepatitis in the post-operative<sup>(21)</sup>.

In the evaluation of the pancreatic function, the potential donors presented alterations in the rate of blood glucose, with hyperglycemia (26.2%) and decreasing in the rate of the amylase enzyme (26.2%). Hyperglycemia is very common in patients with brain death and high dosages of insulin, intravenous, may be necessary for the glycemic control, but it is highlighted that the isolated hyperglycemia and alterations in the isolated amylase are not contraindications for the donation of the pancreas<sup>(14,21)</sup>.

The control of the hyperglycemia must be made through the serial dosages of the blood glucose. In case there is not the possibility of having those dosages, the nurse must guide the team to have the control of capillary blood glycemia, at least, every 4 hours or at smaller intervals when there is a persistence in the disorder<sup>(1)</sup>.

Concerning serology, only 10.0% presented the positive result, and alterations of this type are characterized as absolute contraindication to the donation of organs and tissues, when it is the case of virus of human immunodeficiency and the human lymphotropic virus. Except for the non-melanoma skin cancer and some primary tumors of the central nervous system, the malign diseases make the donation of organs unfeasible<sup>(8)</sup>.

## Conclusion

There was predominance of exams of blood typing, hematology, electrolyte and renal function. Important exams such as the serological tests and the cardiac, hepatic and pancreatic function were not made in a good part of the potential donors. Regarding the laboratorial alterations, the potential donor presented leukocytosis, hypernatremia, alterations of creatine kinase and of creatine kinase -MB,

alterations in the markers of urea and creatinine, in the Glutamic oxaloacetic transaminase and Glutamic-peruvic transaminase, hyperglycemia and decreases of the amylase.

As observed, there are several factors which can contribute for the unfeasibility of the transplantation, among them the laboratorial alterations caused by brain death. So, it is extremely important to have a criterious evaluation of the potential donor aiming at early detecting alterations and treating them.

The knowledge of laboratorial alterations by nursing team provides a better assistance to the potential donor, qualifying the maintenance of the organs and tissues for transplantation. The nurse must be prepared to take adequate measures, together with his team, once the nursing team assumes the responsibility of care of these patients.

Considering the unbalance between the waiting line for transplantation and the number of organs and tissues available for donation, it is fundamental to have the effective maintenance of the donor, thus contributing for the effectiveness of the donations and reduction of the complications and losses of potentially feasible organs.

It is worth highlighting the need of educational activities and continuous training with the health professionals in the hospitals, aiming at improving the assistance to the potential donor, as well as providing studies which evaluate the influence of educational and/or assistential interventions in the effectiveness of donation.

## Collaborations

Torres GV and Freire ILS contributed for the conception, analysis, interpretation of the data, writing of the article and final approval of the version to be published. Vasconcelos QLDAQ and Araújo RO contributed for the collection, analysis, interpretation of the data and writing of the article. Melo GSM and Costa IKF contributed for the statistical analysis of the data, interpretation of the data and final approval of

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