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COVER: Mesenchymal stem cells labeled with fluorescent molecules.

Association of Dental Pulp Stem Cells and Ricinus Bone Compound in a Model of Bone Defect

Alan Araújo de Jesus[1,2], Adriano Araújo de Jesus[1,2], Flávia Oliveira de Lima[1,3], Luiz Antônio Rodrigues de Freitas[1], Cássio Santana Meira[1,4], Ricardo Ribeiro dos Santos[1,2,4], Milena Botelho Pereira Soares[1,2,4*]

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Autogenous bone grafting is needed in some bone tissue defects; however, it causes secondary surgical wounds and morbidity. Tissue bioengineering may be an alternative approach for bone regeneration. Here we investigated the osteogenic potential of dental pulp stem cells from deciduous teeth (DPSC) in association with a Ricinus bone compound (RBC) in a model of bone defect. The influence of the biomaterial RBC on the proliferation and osteogenic differentiation of DPSC was assessed *in vitro* by MTT metabolism and alizarin red staining, respectively. The morphologic analysis was performed using the optic and scanning electron (SEM) microscopies. For the *in vivo* study, 54 Wistar rats submitted to calvarial defects were filled with RBC or RBC+DPSC. A control group had the defects filled only with blood clots. Analyses were performed 15, 30 and 60 days after treatment using digital radiography, optical microscopy, SEM and chemical analysis by electron dispersive spectroscopy. The Ricinus bone compound (RBC) did not inhibit the osteogenic differentiation *in vitro*. No spontaneous regeneration was observed in the control group. The area of the calvarial defect of the RBC+DPSC group showed greater radiopacity on day 15. The RBC presented no reabsorption, was biocompatible and showed osteointegration, working as a mechanical filling. Only sparse ossification areas were found and those were larger and more developed on the RBC+DPSC group when compared to animals treated only with RBC. RBC in association with DPSC is a promising combination for applications in bone regeneration.

Keywords: Bone Defect. Dental Pulp. Stem Cells. Tissue Therapy.

Abbreviations: CV: coefficient of variation; DMEM: Dulbecco's modified Eagle medium DPSC: dental pulp stem cells; EDS: electron dispersive spectroscopy; FBS: fetal bovine serum; MTT: 3-[4, 5-dimethylthiazolyl-il]-2,5-diphenyltetrazolium bromide; RBC: Ricinus bone compound; SEM: scanning electron microscopy.

Introduction

The bone is a very dynamic tissue with good regenerative capacity, but when significant tissue loss occurs due to traumas or injuries, graft replacement is needed. Autogenous grafts, also known as autografts, are the golden standard choice of treatment; however, autograft replacement requires a donor area, leading to an extra surgical wound, increasing consequently time and complexity of the surgical procedure and the risk of infections, worsening postoperative recovery and raising costs. Moreover, there are limitations to obtain accessible donor areas with enough good-quality bone tissue [1]. Biomaterials are an alternative for autogenous bone graft and its inherent problems, and different biomaterials have been used for bone lesion regeneration. The available materials, however, do not have all properties to allow their use in all types of defects [2]. A promising perspective for avoiding the disadvantages of autogenous grafts would be supplying already available biomaterials with cellular components. The absence of cellular components is the main deficiency of this type of material. Combining them to previously collected and cultured stem cells from the patient may generate a cellularized micro-environment similar to a natural bone, allowing total integration, graft vascularization, and, therefore, bone regeneration [3].

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Deciduous teeth pulp is an accessible source of stem cells obtainable from non-vital organs, normally disposed of after the exfoliation phase for the eruption of permanent teeth [4]. Dental pulp stem cells (DPSC) have proliferation capacity and a promising tissue regeneration induction potential previously shown [4,5].

The objective of this study was to assess the *in vitro* and *in vivo* osteogenic potential of DPSC when associated with the biomaterial Ricinus bone compound *in vitro* and *in vivo* in a model of bone regeneration in rats.

Materials and Methods

Biomaterials

We used in the study the biomaterial Ricinus bone compound (RBC; Poliquil Araraquara Polímeros Químicos, Araraquara, Brazil), a polyurethane composed of a pre-polymer derived from isocyanate, a polyester polyol derived from castor oil and calcium carbonate (0.65/1) and calcium carbonate 50% (which allows the formation of pores and gives a better standard of resistance and elasticity in comparison to the bone tissue), with granule particles of about 500 µm. To assess the biomaterial by scanning electron microscopy (SEM), a JSM 6390LV microscope (JEOL Ltd., Tokyo, Japan) was used. Particles of the biomaterial were bound to stubs with carbon fiber and subjected to a sputter coating device SCD 050 (BAL-TEC AG, Liechtenstein, Switzerland) for deposition of an ultrathin layer of gold, turning the samples electrically conductive.

Dental Pulp Stem Cell (DPSC) Culture

We used a previously characterized human cell line obtained from pulp tissue removed from a deciduous tooth extracted near the natural exfoliation phase [6]. DPSC were cultured in tissue culture flasks containing Dulbecco's modified Eagle medium (DMEM; Life Technologies, GIBCO-BRL, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS; Cultilab, Campinas, Brazil) at 37º C and 5% CO_2. The medium was renewed every three days during approximately 10 days when the culture reached 80-90% confluence. Cells were detached using 0.25% trypsin (Invitrogen Corporation, São Paulo, Brazil). After washing with serum-free medium, cell suspensions were counted using a hemocytometer and used for *in vitro* and *in vivo* studies.

In vitro Study

Assessment of Cell Proliferation

An extract of the biomaterial was obtained after incubation in DMEM (200 mg of biomaterial for 1 ml of medium) for 24 hours at 37ºC. After incubation, extracts were subjected to centrifugation for collection of the supernatant. DPSC (10^4/well) were plated in 96-well plates. Cells were incubated for 24 hours (5% CO_2, 37°C, > 90 % humidity), allowing them to adhere and form a semi-confluent layer. Next, cells were incubated in the absence or in the presence of the extract of the biomaterial (diluted in complete DMEM medium at 1:5, 1:100 and 1:250), in triplicate. Qualitative analysis was performed by optical microscopy. Quantitative analysis was done by the MTT (3-[4, 5-dimethylthiazolyl-il]-2,5-diphenyltetrazolium bromide; Sigma-Aldrich, St. Louis, MO) assay, 48 hours later. Plates were incubated for 4 h at 37°C when the reagent was removed and 0.1 N of hydrochloric acid diluted in isopropanol was added. Absorbance was determined 30 minutes and 4 days later using a spectrophotometer Spectramax 190 (Molecular Devices, Sunnyvale, CA) at 570 nm.

Osteogenic Differentiation

For osteogenic induction, DPSCs were cultured in 24-well plates with DMEM medium supplemented with 10% FBS for two days, until 50% confluence was reached. Medium for osteogenic differentiation (DMEM, 10% FBS, 100 nM dexamethasone, 0.05M L-ascorbic acid

2-phosphate, and 10 mM β-glycerophosphate; all from Sigma-Aldrich) was added, with or without the conditioned media of the biomaterial. Cultures were then incubated at 37°C and 5% CO_2 for 21 days. A control culture of DPSC was maintained only with DMEM with 10% FBS. Experiments were carried out in triplicate and the media was replaced every 3 days. Cultures were then washed with PBS, fixed using 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA) for 30 minutes, washed twice with distilled water, and then stained with alizarin red (Sigma-Aldrich) at 2% for 3 minutes. After removal of the dye, cultures were washed three times with distilled water. Digital images of the calcium deposits were used for quantitative analysis, to estimate the percentage of the stained area in each well using the Image-pro program (Media Cybernetics, Rockville, MD).

Assessment *in vivo*

Animals

Animals were provided and maintained at the Animal Facility of Instituto Gonçalo Moniz/ FIOCRUZ. Fifty-four adult male animals (Rattus norvegicus, strain Wistar) were used, weighing between 400 and 450 g, between 5 and 6 months of age. Animals were randomly selected and equally divided into three groups with three time-points each. All experiments followed the regulation for use of animals in experiments and were approved by a local Ethical Committee for Animal Use.

Surgical Procedure

Animals underwent general anesthesia by intraperitoneal injection with xylazine hydrochloride (5 to 10 mg/kg) and ketamine hydrochloride (50 to 75 mg/kg). After trichotomy, the periosteum was detached through a linear incision to give access to the bone tissue. The calvarial bone defect was created in the median portion using a 7 mm trephine drill (FTR06, SIN, São Paulo, Brazil), coupled to a counter-angle with 1:16 reduction, and activated by a low rotational speed surgical motor (Implantmed Si - 923 WH, Bürmoos, Austria), under constant irrigation with sterile saline solution. Grafts were executed at this moment. Three types of treatments were carried out: a control group where defects were filled only with blood clots (control), with 25 mg of RBC homogenized with a blood clot (RBC group) or with 25 mg of RBC homogenized with blood clot plus $2x10^6$ cells (RBC+DPSC group). Animals were euthanized on days 15, 30 and 60 after surgery. Standard size bone fragments of the defects were removed, including a safety margin around the defect. For the removal of bone fragments, a low rotational diamond drill was used under constant irrigation with a saline solution. For histological procedures, the obtained bone pieces were kept in 4% buffered paraformaldehyde for seven days under refrigeration. After fixation, all bone pieces were photographed (100 mm macro lens, Canon Inc., Tokyo, Japan) on its inferior and superior view for registry of integrity and further macroscopic analysis.

Digital Radiographic Analysis

Samples were placed in a transversal position on the image slides for periapical radiographs on the digital radiography system CDR USB (Sirona Dental, Inc., Long Island City, N.Y.). An aluminum penetrometer of 5 degrees with a 1 mm increment was added to the system. The radiographic device (70 kV) was programmed for an exposition time of 0.02 seconds, with a focal distance of 10 cm. The software Photoshop® (Adobe Systems Incorporated, San Jose, CA) was used for the correction of brightness in the images, using the penetrometer as a parameter. To quantify the tissue radiopacity, the mean and standard deviation of the gray intensities was measured using the Image Tool® software (University of Texas Health Science Center, San Antonio, TX). The coefficient of variation (CV) of the same area was also calculated. For each radiograph, two circumferences of the same diameter were established: one on the defect place and the other

outside any area with residual bone tissue. This area was set as an individual negative control for each image. The final result was obtained by subtracting the value from each control area from its defect area. Defect areas were not compared among each other, since there is a difference in contrast between the images, which could create a bias in the absolute value for direct comparison between samples, even taking into account the brightness correction previously performed. To eliminate the possible influence of the biomaterial on the results, radiographs of the biomaterial alone were carried out separately, following the same protocol.

Histological Analysis by Optic Microscopy

After fixation, samples were decalcified in 7% nitric acid for 72 hours. Samples were then transversally cut through their large diameter, subjected to the standard protocol for paraffin-embedded tissue, and sliced with a microtome RM 2125 (Leica Biosystems, Wetzlar, Germany) in 5 µm thick transversal sections. Slices were stained with conventional hematoxylin and eosin staining. Tissue regeneration was observed under an optical microscope with different resolutions and assessments done by descriptive analysis. Several aspects were reported, such as the presence of inflammatory cells, the number of collagenous fibers, contact interface between the biomaterial and bone tissue, degradation of the particles of the biomaterials and osteogenesis.

Scanning Electron Microscopy Analysis (SEM)

The analysis by SEM was carried out in the group of animals sacrificed at 60 days since it was the longest period for bone regeneration studied. For fixation, samples were immersed in 4% paraformaldehyde, 2.5% glutaraldehyde diluted in 0.1 M sodium cacodylate buffer. After fixation, pieces were cut in the middle through the larger diameter of the defect. Half of each sample was decalcified and prepared for optic microscopy and the other half was prepared for SEM. The samples prepared for SEM were subjected to dehydration through a sequence of alcohol baths in different concentrations using a critical point drier (CPD 030, BAL-TEC), and to metallization by a sputter coating, as described above. For comparison, assessment of integer bone fragments from healthy animals was also carried out using the same size as for the defects in the test samples.

Chemical Analysis by Electron Dispersive Spectroscopy (EDS)

Chemical compounds present in the samples were observed using the EDS Nanotrace system TN-JEM1230-3NUS (Thermo Electron Corporation, Waltham, MA). The distance from samples to the detector was 10 mm and 15 kV. Sample preparation followed the same protocol as for SEM.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism® software version 5.01 (GraphPad Software, Inc., San Diego, CA). Mean differences were subjected to variance analysis using one-way ANOVA with the Bonferroni test for multiple comparisons. P-value was considered significant when lower than 0.05.

Results

The biomaterial RBC presented particles up to 800 µm in size, with an irregular shape, broad concavities and spiked edges with a slightly rough surface, except for the areas where fractures occurred during the grinding process (Figures 1A and B). DPSC were cultured in complete DMEM medium in the absence or presence of RBC extract. After two days of culture in RBC extract at 1:5 dilution, we observed a reduction in cell viability. RBC at 1:250 did not inhibit cell proliferation, as shown by MTT assay (Figure 1C). Based on these results, we selected 1:250 dilution of the extract to be used.

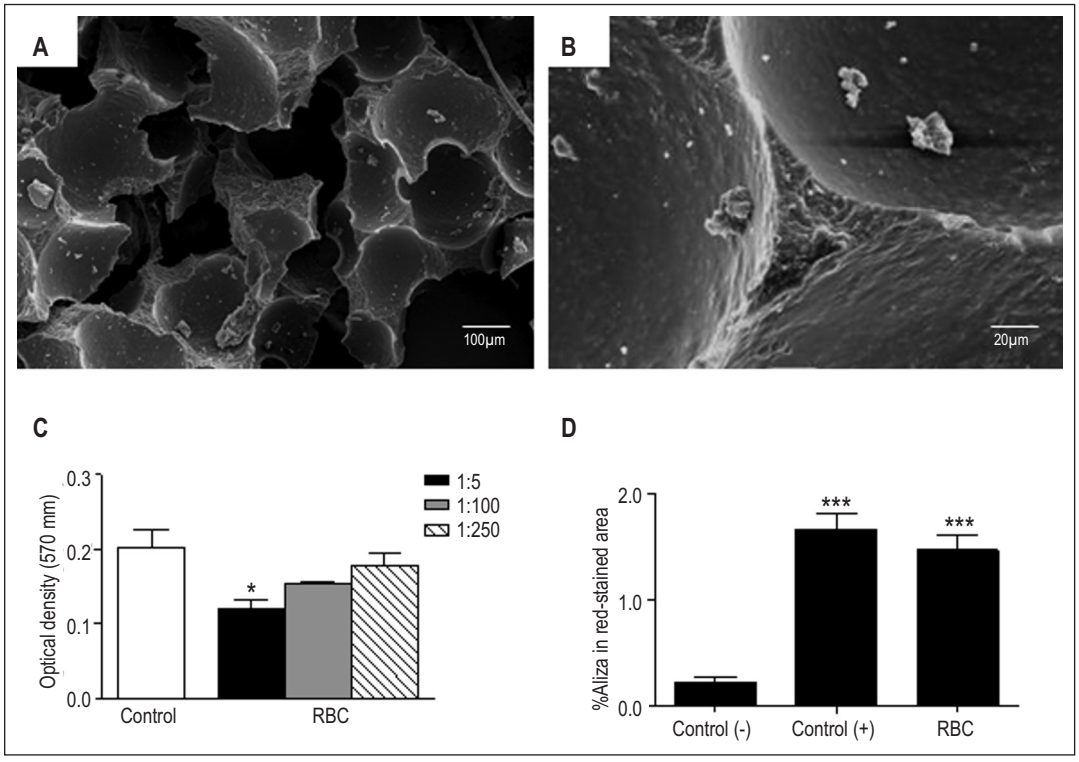
DPSC cultured in osteogenic medium in the absence or presence of RBC extract were analyzed to detect calcium depositions by alizarin red staining 21 days after culture. A statistically significant difference in the percentage of alizarin red-stained area was observed in the groups cultured in the osteogenic medium when compared to the control cultures in DMEM medium alone (Figure1D). No differences between cultures incubated without or with the RBC extract in osteogenic-inducing conditions were found.

The model for cranial vault defect provided areas with a 7 mm diameter with no spontaneous regeneration until the end of the experimental procedures. Macroscopic assessment of the pieces 15 days after defect induction showed a better regeneration of the periosteum and endosteum than in the RBC+DPSC group than in the RBC group, as shown by an increased tissue formation in the regeneration area. The control group had reduced regeneration (Figure 2).

Quantification by analysis of digital X-ray images at day 15 revealed a statistically significant difference in the mean of gray intensity levels (Figure 3A) and coefficient of variation (Figure 3B) among the three groups. The control group showed no radiopacity of the tissue. RBC+DPSC group obtained higher values for gray level and CV than the RBC group. Analysis carried out on days 30 and 60 revealed that all groups increased in value over time, with no significant difference between the gray levels in groups RBC and RBC+DPSC ($p>0.05$), while both groups had significantly higher levels ($p<0.05$) than the control group (data not shown).

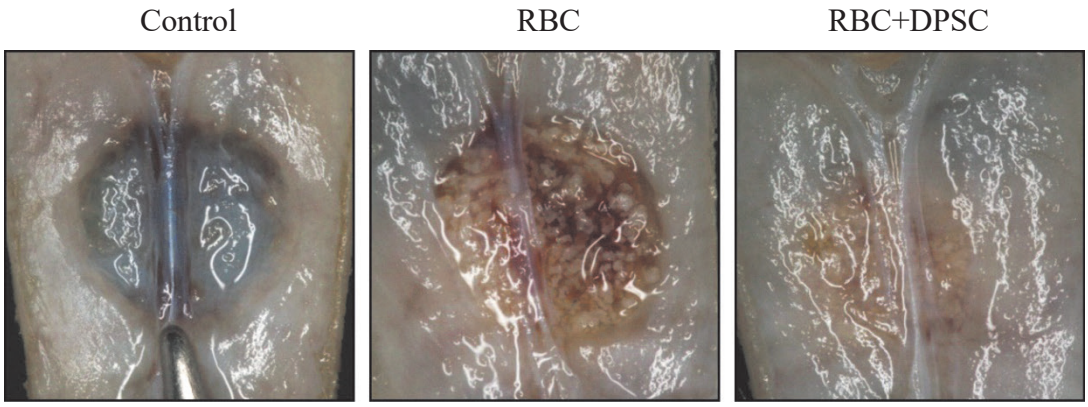
Histological analyses showed a marked decrease in thickness on the defect area in the control group (Figure 4A). This area was filled

Figure 1. Ultra-structural analysis of the biomaterial by scanning electron microscopy.



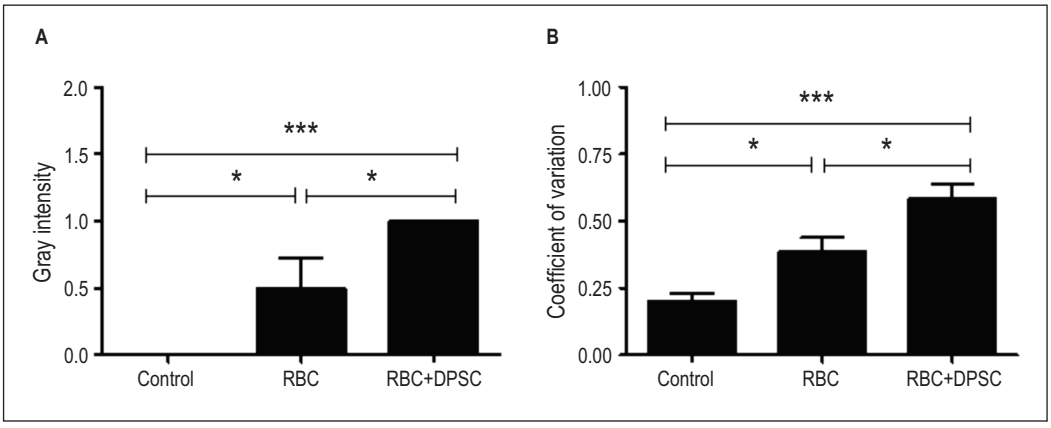
(A) RBC particles of different sizes, with sharp ridges. (B) The detail in higher magnification showing differences in the surface roughness between areas of convexities and areas of fragmentation. (C) RBC at 1:250 did not inhibit cell proliferation, (D) No differences in osteogenic differentiation between cultures incubated without or with the RBC extract.

Figure 2. Macroscopic assessment of the pieces 15 days after defect induction.



(A) Animals where defects were filled only with blood clots (control). (B) Animals where defects were filied with 25 mg of RBC homogenized with a blood clot (RBC group). (C) Animals where defects were filled with 25 mg of RBC homogenized with blood clot plus $2x10^6$ cells (RBC+DPSC group).

Figure 3. Digital radiographic analysis in bone defects. Three types of treatments were carried out: a control group where defects were filled with a blood clot (control), with 25 mg of RBC homogenized with a blood clot (RBC group), or with 25 mg of RBC homogenized with blood clot plus $2x10^6$ DPSC (RBC+DPSC group). Groups of rats were euthanized on day 15 for analyses.



(A) Gray levels of the defect region in the three studied groups. (B) Coefficient of variation of the defect region in the three studied groups. The values represent the mean±SEM from the percentages of 6 animals/group. *p<0.05 and ***p<0.001.

in with fibrous connective tissues presenting fusiform cells arranged horizontally, progressing from loose on day 15 to dense on day 60. Several blood vessels were found near the margin of the defects in all-time points with a greater number at day 60 when vessels were found spread into the center of the defects. Regarding bone regeneration, the defects were critical as they would not regenerate spontaneously. No significant signs of an inflammatory process, either chronic or acute, resulted from the surgical trauma, were found at the time points analyzed (Figure 4A). On the RBC samples, no signs of particle degradation were found at the studied time points, and the presence of the biomaterial kept the thickness of the defect (Figure 4B). The ridges of the particles

seemed to hamper contact between the connective tissue and the material's surface (Figure 4C). Some giant multinucleated cells were sparsely found making contact with some particles; however, no inflammatory infiltrate was found, suggesting biocompatibility of the material. Regarding bone regeneration, ossification of some areas was occasionally found at days 30 and 60 (Figure 4D). In those regenerating areas, it was possible to identify a layer of osteoblasts surrounding the region and osteocytes in their center, suggesting regeneration progression was taking place. We observed osseointegration between the particles of the biomaterials and newly formed bone tissue in the groups at day 60 (Figure 4E) and the presence of mineral tissue in close contact with the particles of the biomaterial. Blood vessels were found spread widely over the defect in all-time points, and in a greater frequency at day 60. The characteristics of the RBC+DPSC group were similar to the RBC group, yet larger ossified regions with maturation aspects were found already on day 15 (Figures 4F and G). In both groups where the biomaterial was used, it was possible to correlate ossified regions with the proximity of a larger number and blood vessels, especially those of larger calipers. Bone regeneration was restricted to incidental (random) areas and most particles were still surrounded by fibrous connective tissue (Figure 4H).

The analysis by SEM enabled assessment of the microarchitecture of the defects, and validation of the findings observed by optic microscopies, such as a decrease in thickness on the defect area in the control group (Figure 5A) and the recovery of the thickness by the biomaterials (Figure 5B), properties of the edges (Figure 5C), bone regeneration (Figure 5D) and distribution of blood vessels (Figure 5E). Samples exhibited tissue with fibrous aspect portraying the lack of tissue regeneration. However, regions where fibers were arranged in bundles, which might have progressed to bone tissue over time, were also found (Figure 5F).

At day 60 the biomaterial did not sustain changes in its form after making contact with the tissue, and the particles kept their aspect, with spiked edges, hampering a full contact between the material's surface and the newly-formed tissue (Figure 5G). The assessment of intact bone fragments showed that their thickness (Figure 5H) were similar to those of the groups with biomaterials, confirming their role as filling material.

The EDS analysis determined the spatial distribution of chemical elements and confirmed the presence of areas of bone regeneration (Figure 6). In the control group, there were no areas of bone regeneration in any of the samples, while in groups RBC and RBC+DPSC calcified nodules were identified sparsely distributed over the defect area.

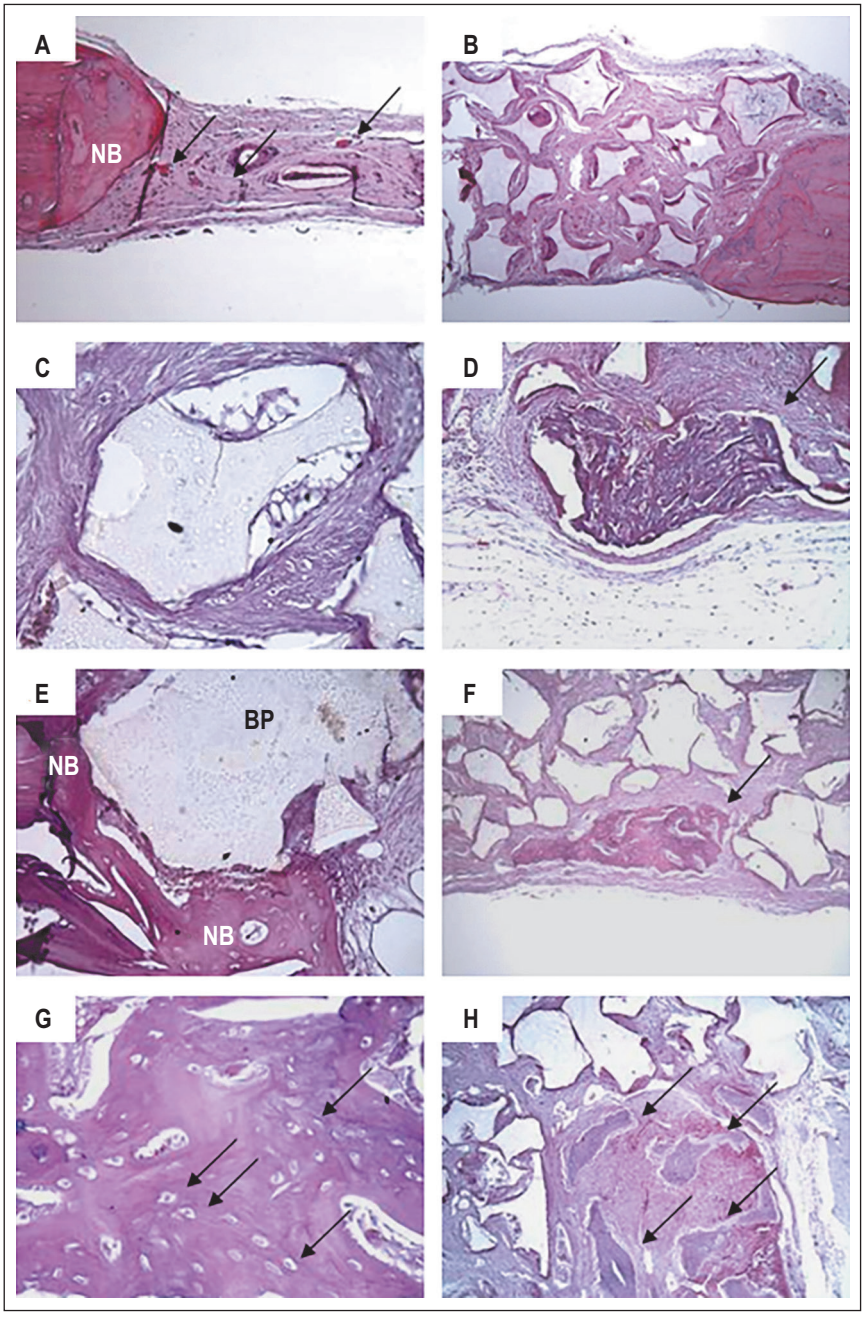
Discussion

There is a constant search for replacements of bone tissue to treat bone defects and lesions. In this context, the development of biomaterials and techniques of cell therapy raises promising perspectives in this area. Several studies have been published reporting bone reconstruction using stem cell transplantation [5,7]. The ideal replacement would be a biomaterial with osteoconductive and/or osteogenic and/or osteoinductive properties [8]. In the present study, we evaluated the biomaterial RBC in association with DPSC.

The choice for DPSC was mainly due to promising results reported [4,5,9], where the capacity of proliferation, plasticity, and tissue regenerative potential of DPSC was confirmed. However, it should be emphasized that the collection of the tissue from decidual teeth has a limited time due to mixed dentition [10]. A way to bypass this limitation is the cryopreservation of the cells, the pulp tissue, or even the whole tooth [11].

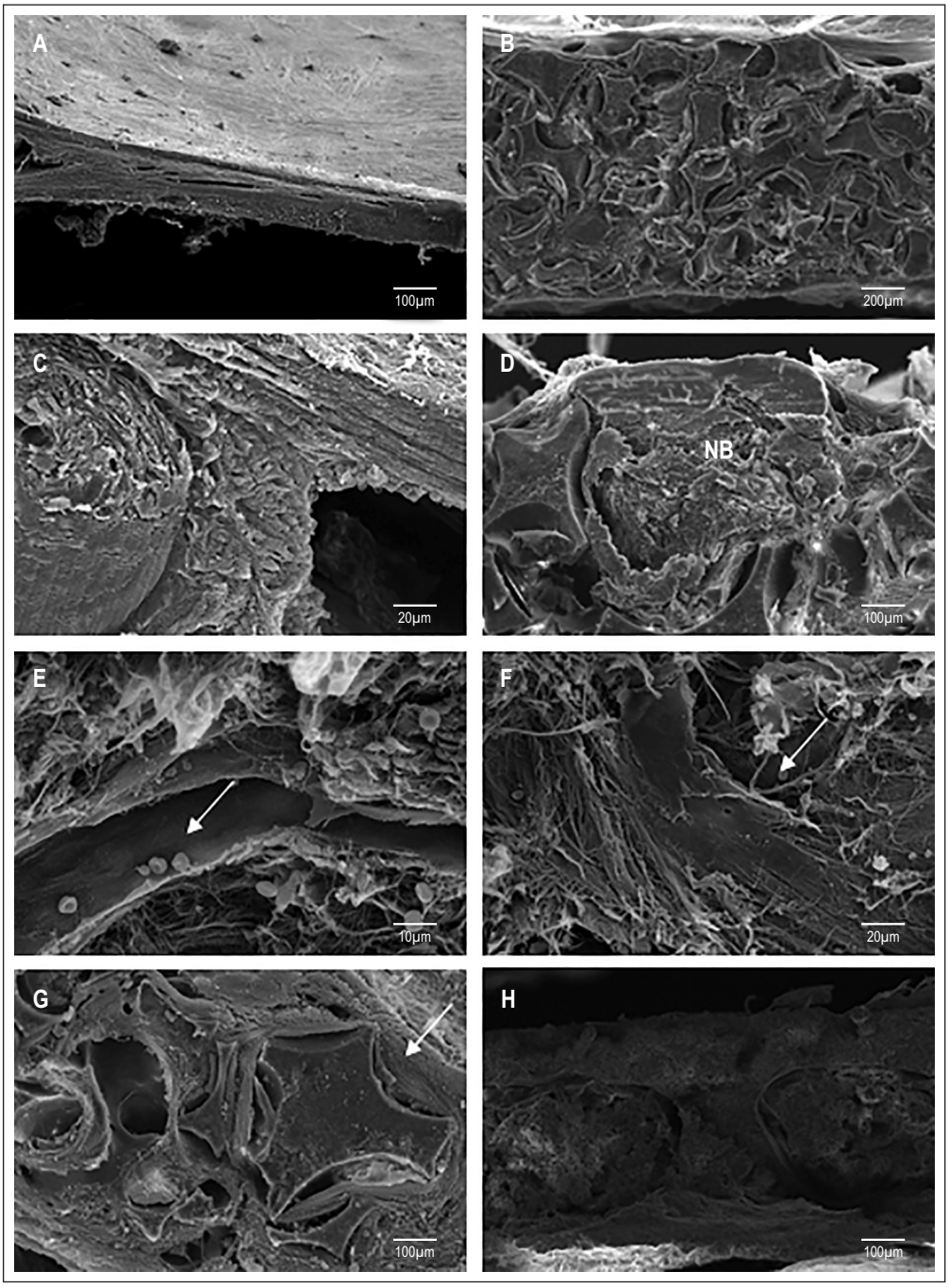
Calcium deposition was observed in all cultures using osteogenic medium and biomaterial extract, confirming the differentiation process. It is a positive result that the biomaterial did not inhibit cell differentiation. This work did not assess the potential of biomaterials for inducing differentiation in undifferentiated stem cells. However, Beloti

Figure 4. Histological analysis of bone defects by optical microscopy.



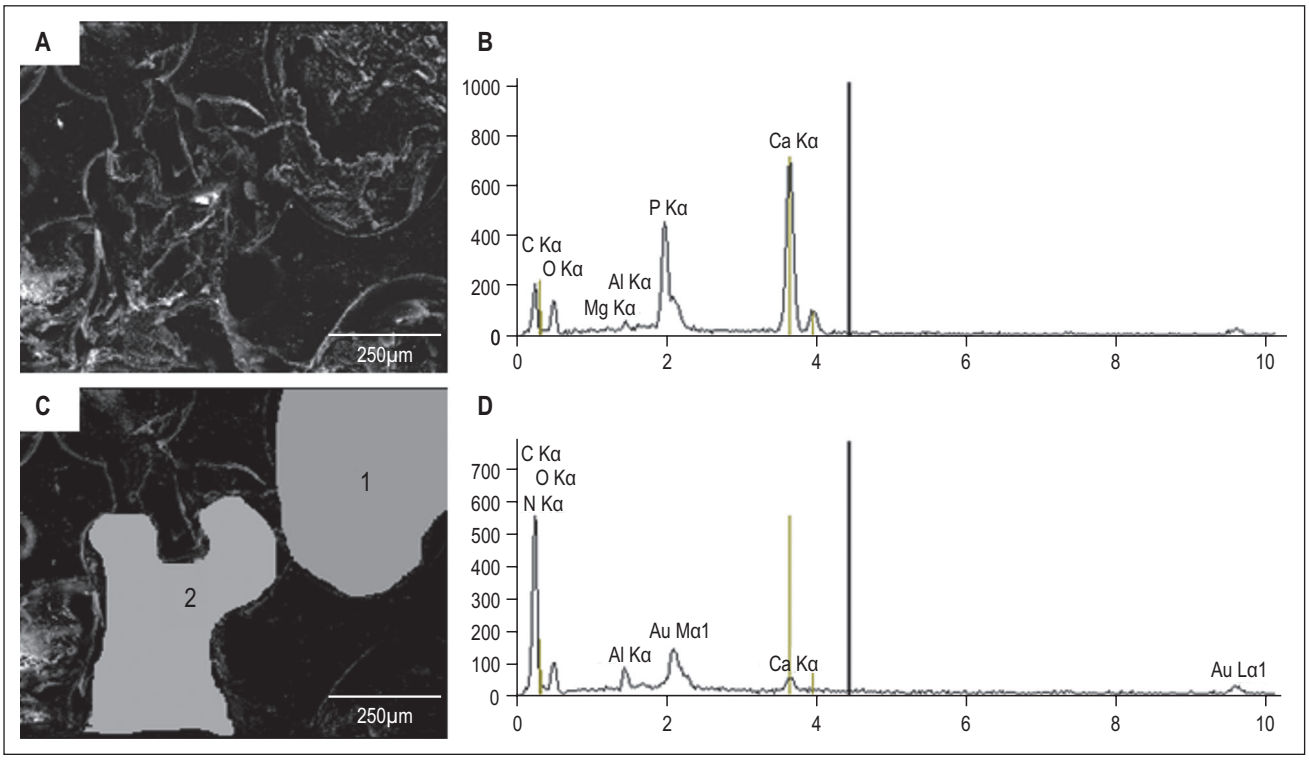
(A) The section made from one animal from the control group euthanized at day 60 after defect induction, showing a discrete bone neoformation restricted to the margins (NB), with a decrease in thickness in the defect region. Blood vessels near the edges (arrows). (B-E) Section obtained from animals of the RBC group. (B) Maintenance of the anatomy before the defect at day 30 after defect induction. (C) Particles of biomaterial with spiked edges blocking contact from the tissue with the surface of the biomaterial at day 60 after defect induction. (D) Presence of calcification points in the center of the lesion (arrow) at day 60 after defect induction. (E) Signs of osseointegration of the biomaterial's particles (BP) with the newly-formed bone (NB) at day 60 after defect induction. (F-H) Sections were obtained from the RBC+DPSC group. (F) Calcification area in the center of the lesion (arrow) at day 15 after defect induction. (G) Details of figure F, showing the maturation aspect of the tissue, with the presence of several osteocytes (arrows). (H) The region with multiple individual calcification areas (arrows) 30 days after defect induction. Hematoxylin and eosin staining. Obj: 10x (A and B), 20x (F and H), 40x (D) and 60x (C, E and G).

Figure 5. Ultra-structural analysis by scanning electron microscopy at day 60 after bone defect induction.



(A) Details of the decreased thickness of the bone defect in the control group. (B) View from the bone defect thickness, showing the mechanical filling role of the biomaterial in section obtained from the RBC group. (C) Lesion margin with rounded aspect with no signs of bone neoformation in section obtained from the control group. (D) Presence of calcified region (NB) in section obtained from the RBC+DPSC group. (E) Longitudinal section of a blood vessel with erythrocytes (arrow), demonstrating vascularization, but with the surrounding tissues not calcified in the sample from the RBC group. (F) Fibrous aspect from the tissue in the center of the bone with bundles of newly formed fibers (arrow) in the sample from the RBC group. (G) The contact surface between newly formed fibers and the edges of the material (arrow). (H) Micrograph of the intact bone fragment at the same studied area.

Figure 6. Comparison of bone regeneration between two selected areas in one of the RBC+DPSC samples by electron dispersive spectrometry.



(A) Micrograph of the entire analyzed area by SEM. (B) Quantitative analysis of the chemical elements present in area 1. (C) The two selected areas (in gray) for analysis do not contain biomaterial particles. (D) Quantitative analysis of the chemical elements present in area 2. A bone neoformation in area 1 is confirmed by its chemical composition.

and colleagues [12] found no signs of osteogenic differentiation in cultures associated with polyurethane discs derived from *Ricinus communis*.

The choice for the RBC biomaterial was due to its vegetal origin, which avoids possible complications related to the use of biomaterials from human and animal sources, such as contamination and resistance to the user due to the patient's principles. Also, RBC is derived from castor oil, a low-cost renewable resource found in abundance in Brazil and other countries [13]. Moreover, there is no *in vivo* report assessing its association with DPSC in bone regeneration.

The analysis by digital radiographs showed that the control groups presented significantly lower values of gray levels and CV than the groups with the biomaterial, proving the biomaterial's role as a mechanical support for the regenerating tissues. Results of gray level intensities express an increased general radiopacity of the area, while CV indicates a greater heterogeneity of the analyzed tissues. The analysis at 15 days demonstrated higher gray levels and CV values for RBC+DPSC groups than for the others ($p<0.05$). RBC+DPSC groups showed more radiopaque pixels that may suggest early-onset bone regeneration, possibly due to the influence of DPSC on the tissues. This increase in radiopacity was also observed by Del Carlo and colleagues [14], in animals that received polyurethane from castor oil associated with aspirate from autologous bone marrow to regenerate bone defects.

Confirming these results, macro and microscopic assessment of this group at the same time point presented also improved regeneration. However, it is worth mentioning that radiograph analyses have limitations and it is not possible to assert the type of tissue formed in the bone defects, although

radiographic analysis can be found in several reports on bone regeneration [15]. The samples may become more radiopaque due to an increase in soft tissue instead of bone tissue formation. In any case, a potential tissue formation may represent a regenerative activity, which may be confirmed and better understood when investigated in combination with microscopic analyses, as carried out in this study.

Association of analysis between optical microscopy and SEM allows assessment of the histological characteristic and tissue microstructures, identifying ossified regions and the interaction between the tissue and the biomaterial [16]. Although the assessment by SEM was qualitative, in case of doubt whether a structure was truly the result of bone regeneration, we performed analyses by EDS to identify and quantify the chemical elements present in the structure and whether those elements present in the right proportion corresponded to regenerating a bone tissue.

The biomaterial fulfilled the basic requirements for biocompatibility [16]. No significant inflammatory infiltrate was found in the samples and it served as support for newly-formed tissues, allowing the defect region to keep its architecture. It was evident the difference in tissue volume between the control group and the groups with the biomaterial. Some regeneration areas showed interaction with the particle's surface of the biomaterial, indicating some type of influence of the biomaterial on the process. It was also detected a close contact between them, possibly meaning a potential for osseointegration. The action of the biomaterials' surface over bone regeneration is well described in the work of Olivier and colleagues [17]. The irregular shape of the RBC particles may hamper bone regeneration due to a decrease in the contact surface, where empty spaces without tissue formation would remain, as suggested before [2].

When comparing RBC+DPSC and RBC groups, samples showed very similar characteristics, though qualitative differences were found. In the group RBC+DPSC, ossified areas happened earlier, from day 15 on, and exhibited larger size at certain points, which may indicate a positive effect of the DPSC on the initial tissue regeneration. As previously reported by Giannoni and colleagues [18], cells could have a regenerative activity for a limited time, decrease over time due to the low cellular level of bone tissue and the difficult tissue vascularization.

An increased vascularization over time was observed in all groups; however, the defect that received biomaterial presented blood vessels overall in all-time points, while in the control group vessels were found predominantly near the margin. This is probably a positive factor, though there is no guarantee of bone regeneration, as previously reported by Koob and colleagues [19].

Although there is a lack of consensus about the ideal degradation time of biomaterials, they ideally should be gradually replaced by the newly-formed bone tissue or incorporated into the tissue's receptor [17]. In our study, the RBC particles did not undergo any volume alteration. This finding is in line with most reports about polymeric biomaterials derived from castor oil [14].

Nevertheless, one report showed signs of particle reabsorption and replacement by bone tissue [20]. The time-frame of the present work did not allow to state that the biomaterial has a null degradation, nor it allows to state that it is slower than the ideal since full regeneration of the effect did not take place. Maintenance of the biomaterial at the defect site might be an advantage in situations as craniofacial bone defects since maintenance of the graft volume is important for esthetical purposes.

Conclusion

Finally, we conclude that the biomaterial tested in this study show biocompatibility *in vitro* and *in vivo* and promising use in association with DPSC. Further studies should be carried out to extend the knowledge of RBC applications in regenerative medicine.

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Development of an Automated Device for the Procedure of Blood Transfusion in Newborns

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Hemolytic disease of the newborn, also known as hemolytic disease of the fetus and newborn, HDN, HDFN, is a problem of fetal erythrocyte hemolysis. This may happen due to the sensitization of maternal antibodies through the placental route. It is the pathology most frequently found in neonatal patients. Approximately 98% of maternal alloimmunization cases by erythrocyte antigens are due to the RhD factor. Phototherapy is the first choice in the treatment of neonatal jaundice. Blood transfusion is the therapy instituted for the treatment of severe neonatal disease. This study aimed to develop an automated device for performing the blood transfusion procedure. The method was developed in four stages: (1) Literature review about the search for theoretical references based on scientific articles and textbooks on transfusion therapy in the newborn; (2) Elaboration of the device consisted of items related to its assembly and structuring; (3) Operation of the medical device, including specific schedules related to the execution of the procedure; (4) Performing tests with simulating the purpose of volume replacement in the total cycle performed in the exsanguine transfusion procedure. The results showed that it was possible to assemble, reproduce, and implement the automation of the device developed for the exsanguine transfusion procedure in a practical. Also, the procedure presented security and effectiveness in the clinical treatment related to HDN.

Keywords: Newborn Hemolytic Disease. Fetal Erythroblastosis. Neonatal Jaundice. Kernicterus. Total Transfusion.

Abbreviations: HDN: Hemolytic disease of the newborn. HDFN: Hemolytic disease of the fetus and newborn. Rh(D): Rhesus factor D.

Introduction

Fetal erythroblastosis or hemolytic disease of the newborn is a typical disease caused by the incompatibility between the Rh Factor of maternal and fetal blood. In this pathology, the baby Rh factor is decisive, and the Rh factor of the mother is negative, which leads to the production of anti-Rh antibodies in the maternal organism to combat the Rh agent of the fetus. Currently, even with the introduction of anti-Rh (RhIg) immunoglobulin, Rh (D) incompatibility still happens [1].

Among Caucasians and Afro-Americans descendants in the United States, the incompatibility concerning Rh (D) antigen occurs in approximately 10% of all pregnancies; 60%-70% of Rh (D) negative women happens with an Rh (D) positive baby. Before the routine use of Rh Immune Globulin (RhIG) prophylaxis in obstetric practice, approximately 15% of D-negative women develop anti-D. Anti-D is still one of the most common antibodies associated with HDN, occurring around 1 in 1,000 births, although the rate has decreased dramatically since 1968 with the introduction of RhIG. These statistics reflect the decline in the incidence of anti-D, but they underestimate the current contribution of HDN because early intrauterine deaths are not detected, whereas neonatal deaths can be attributed to other prevalent complications [2].

Also, during pregnancy, it is recommended to identify the maternal blood type for the ABO and RhD system, and screen if irregular anti-erythrocyte antibodies are being produced. The diagnosis of isoimmunization by the Rh system is made based on the detection of anti-D antibodies in maternal serum. The Indirect Coombs test is the gold-standard method for diagnostic purposes,

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as it is the most accurate to determine antibody titers [3].

RH isoimmunization begins to be studied based on previously obtained data and laboratory tests identify the presence of antibodies in the mother's blood. Treatment for isoimmunization will depend on the degree of anemia of the fetus. Nevertheless, the pregnant woman must perform the first follow-up in her prenatal care, and also to measure the possible severity of the problem in order to schedule the delivery and avoid severe fetus' sequelae.

Phototherapy is the first line of treatment for neonatal jaundice and can prevent blood transfusion. The blood transfusion is necessary when phototherapy fails to promote an adequate reduction in bilirubin levels or when the initial serum bilirubin is so high that it implies a high risk of the newborn develops Kernicterus [4].

Innovative activities in the health area are characterized by a strong interaction with the scientific industry. On the one hand, the scientific infrastructure is the source of a flow of information that supports the emergence of innovations that affect medical practice and health such as new drugs, new equipment, new clinical procedures, new prophylactic measures, and further knowledge. Still, medical practice and the performance of the health sector are the source of an inverse flow of information and constitute a vast and growing repository of issues, empirical findings, and successful practices that need to be explained and understood [5].

So, this study aims to discuss the topic of HDN to present the characteristics of the disease, therapeutic solutions used, as well as the presentation devices that can assist the treatment of exsanguine transfusion using innovative methods associated with technologies in health [6]. The update in this field seeks to add safety to the procedures and confidence of the patients associated with a lower psychological, exhaustive, and manual burden for health professionals.

Literature Review

History of Immunohematology in the Discovery of Hemolytic Disease in the Newborn - HDN

Although changes in the fetus and newborn have been observed since the 17th century concerning issues oh HDN, the theme highlights in 1939 when Levine and Stetson published the results from a transfusion reaction of the husband's blood to a woman after delivery. These authors postulated that the mother had been immunized against the fetus' antigen by the father. In 1940, Landsteiner and Wiener carried out experiments to immunize rabbits and guinea pigs against erythrocytes of rhesus monkeys. Using the serum from these experiments, Levine demonstrated that the mother who had suffered the transfusion reaction was rhesus-negative, and the father, rhesus-positive. Also, the mother's serum agglutinated the father's erythrocytes [7].

Few studies analyze the incidence of perinatal hemolytic disease in Brazil, a condition caused by the hemolytic action of maternal antibodies against fetal red blood cell antigens, which results in fetal and neonatal hemolytic anemia. Although the introduction of anti-Rh (D) immunoglobulin in clinical practice has considerably reduced the incidence of the disease, some evidence suggests that the number of cases is still high, even in developed countries [8].

Hemolytic Disease of the Newborn - HDN

The transplacental passage of antibodies against fetus blood cells from the 10th week of gestation can cause premature destruction of these cells (hemolysis), leading to fetal anemia. This condition is called HDN. HDN is the most complex of the clinical forms of IgG-mediated hemolysis because it involves the production of antibodies in one individual and cell destruction in another. Normally, the maternal and fetal blood systems do not mix, but transplacental maternal-fetal bleeding can occur. This bleeding

happens most of the time during childbirth, but it can occur spontaneously during the pregnancy, especially in the 3rd trimester or after invasive procedures or miscarriages [3,5]. HDN can be classified as a mild, moderate, or severe condition depending on its clinical manifestations. It is a mild disease because if it presents mild anemia with or without jaundice. In moderate cases, the child has severe anemia and jaundice and may present hepatosplenomegaly, edema, and pallor. In severe cases, there is intrauterine death from liver dysfunction and hydrops fetal. The diagnosis can be made or during pregnancy or after birth. The results are concluded by tests such as the search for antibodies fixed on the red blood cells (Direct Antiglobulin Test - TAD); elution that serves to remove the antibodies present in the erythrocyte membrane to identify them later, serum antibody testing (Irregular Antibody Research - Indirect Coombs or PAI), among others [9].

Phototherapy

Phototherapy is a therapeutic used to treat several dermatoses [10]. Used for more than 50 years, phototherapy does not have a list of still known photodegradation mechanisms, but it is quite widespread and accepted. Its use must be concomitant with laboratory evaluations, in order to highlight the cause of hyperbilirubinemia, which is a frequent clinical problem in the neonatal period. Phototherapy increases the degradation of bilirubin in the skin by photooxidation. It is contraindicated for patients with elevated conjugated bilirubin (Figure 1). Jaundice on the skin is not a reliable means of obtaining serum levels. However, there are also treatment alternatives such as blood transfusion and the use of medications capable of accelerating metabolism and its excretion [11]. The complications caused by the use of phototherapy and its side effects are skin rash, burns, thermoregulation, effects on the retina, effects on the gastrointestinal tract, effects on the speed of cerebral blood flow, behavioral changes, and effects on the baby-family relationship and the bronze baby syndrome. In the case of Rh and sometimes ABO incompatibility, phototherapy (Figures 2 and 3) should be used as an adjunct, and blood transfusion is the most appropriate treatment [12].

Blood Transfusion

Blood transfusion was the first successful therapy to treat severe neonatal jaundice. Although blood transfusion is considered a safe procedure, it is not without risks and has mortality rates ranging between 0.5% and 3.3%. The bilirubin levels to indicate blood transfusion remains controversial since the incidence of bilirubin encephalopathy also depends on other variables such as gestational age, the presence or absence of hemolysis, and the newborn's clinical condition. Current recommendations for performing blood transfusion are based on the balance between risks for the occurrence of encephalopathy and adverse events related to the procedure [13]. The decision to initiate blood transfusion is due to the urgency of lowering circulating bilirubin values and promoting the turnover between them and the tissues to not affect the newborn's central nervous system [14].

Adverse events may occur after the procedure. However, it is not certain that all clinical conditions can be considered adverse events attributed to the procedure, since, in most cases, such events occurred in patients with unstable clinical conditions before blood transfusion [15].

Bilirubin Encephalopathy (Kernicterus)

The pathogenesis of kernicterus is highly complex, and its risk is related to many factors, such as the low water solubility of bilirubin and its tendency to undergo aggregation and precipitate the physiological ph (acid), the ability of bilirubin to freely cross the barrier hematoencephalic, together with prematurity, as well as metabolic acidosis, hypoglycemia, hypothermia, and sepsis can aggravate the neurotoxicity of bilirubin even at lower levels [16].

Figure 1. Bilirubin metabolism of the newborn and fetus.

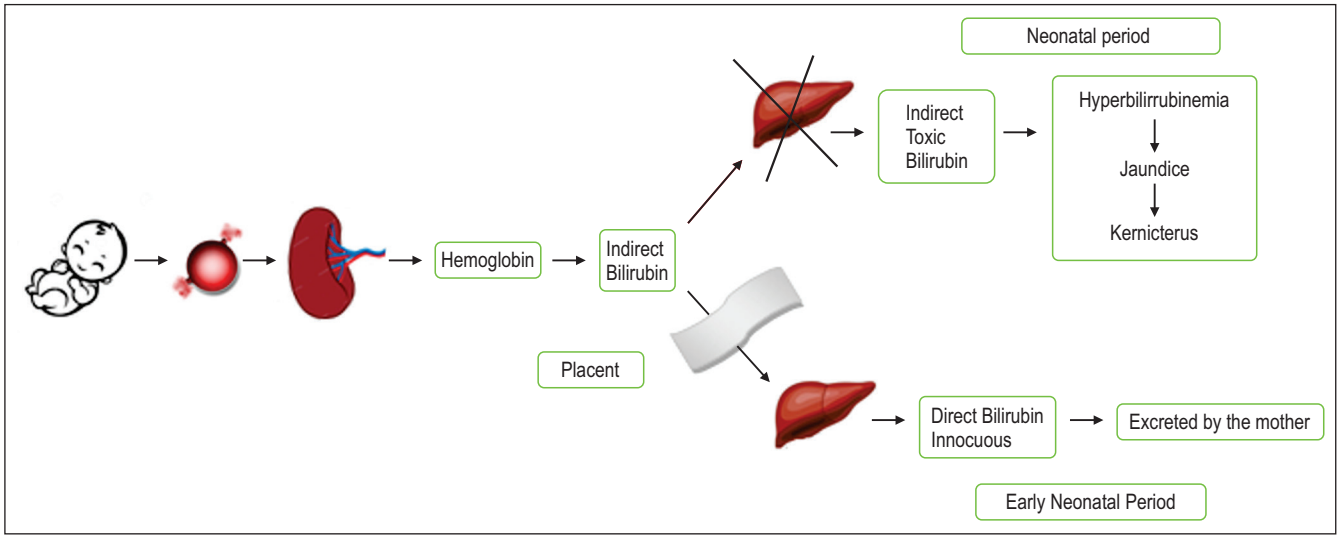
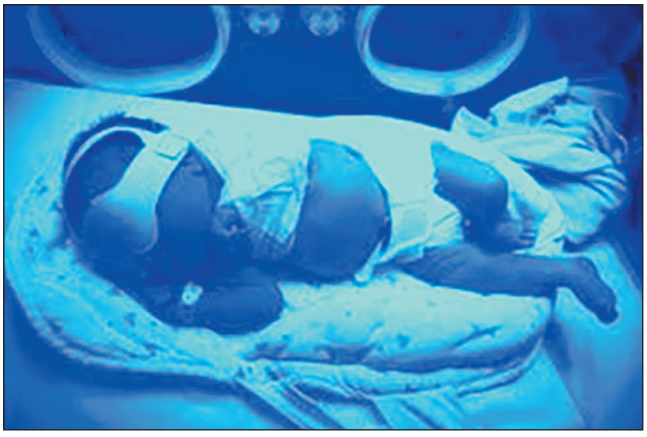


Figure 2. Newborn using portable phototherapy.

Figure 3. Newborn in the use of phototherapy in incubators.



Innovation and Technology in Health

Technology can be generically defined as applied knowledge. In health areas, this applied knowledge allows the prevention, diagnosis, and treatment of diseases and the rehabilitation of their consequences [17]. The health field has been sensitive to technological incorporation for therapeutic, diagnostic, and life maintenance purposes, using the knowledge and products of computer science, new equipment, and materials. However, it has been less aggressive in the use of innovations of the non-material type, especially of the innovations in the field of the organization and work relations [18]. Technology is not only seen as a tangible product but as a result of work that involves a set of abstract or concrete actions that have a specific purpose in health care [19]. For a better understanding and development of this innovative device, it was necessary to study theoretical concepts relevant to the manuals procedures used today to perform the transfusion exsanguine technique. Although some of the concepts require a more extensive detail of the theme for its full understanding, to maintain the main focus, only the features most related to the device were presented here.

Materials and Methods

This study was divided into four phases:

1) The selection of published scientific articles related to the topic in Portuguese and English, and exploratory analysis for the materials' contents (articles that addressed other pathologies associated with HDN were excluded from this work).
2) The development of the prototype related to the automated device for the procedure of blood transfusion.
3) The elaboration of the device and its essential materials such as disposable bags of blood components, equipment, syringes, "tree-ways", as well as a 3D printer of small supports, bearings, and specific compartments for positioning, adaptation, and fitting of these referred materials. In this phase, the device was inserted in the experimental bench to simulate the infusion of blood in the newborn. The programming of the rotation of the "tree-ways" valves related to the process of infusion and disposal left the theoretical conception of the system for practical experimentation, allowing not only the evidence of the functioning of the device but also the programming of the infusion time and the previous determination the volume of the infused blood component. Positively, the concern regarding safety in the correct disposal of the blood component generated at the end of the blood transfusion procedure was aroused.
4) Tests were performed with the system. They were processed with the inclusion of bags of blood components adapted to the system, where inside there were colored liquid for a reliable demonstration of the procedure and its functioning. Also, START / ON, PAUSE, and STOP programs were developed and included for the device's operation and safety, in case of possible intrinsic complications, which could occur during the automated procedure, ensuring the integrity of the newborn.

Record

The device's registration initially began with the application to the Center for Technological Innovation (NIT) of the Bahiana School of Medicine and Public Health. In response, the NIT approved the "Intellectual Protection Feasibility Opinion", now entitled "Automated blood transfusion device". According to the opinion in the thorough search for priority, a total of seven patents were found associated with the chosen theme, which could be registered as processing to perform blood transfusion, but not for the same purpose. Of the evaluated works, only one was identified as a control system in the activity related to the automation of the blood transfusion procedure [patent number 6 (PI 0404065-1 A). Filing Date: 9/21/2004. dissertation].

Conception

The experimental device described in this study is developed as an alternative infuser with four cycles that can replace the manual work currently carried out by the medical team in the blood transfusion procedure. The main purpose of this device is to ensure that the volume and speed of infusion and blood withdrawal of the patient are uniform and that the sequencing of the valves is performed with optimal reliability. These guarantees protect the patient concerning non-collapse (narrowing of the blood vessel walls when subjected to a negative pressure) of the umbilical vein of access, hemolysis in the blood infusion, and reduction of the time to perform the procedure. The prototype was initially tested with bags containing pigmented water with different colors, facilitating the visualization of the "blood" exchange.

Detailed Device Composition

The experimental device consists of a mechanical structure (fixed and mobile part) in a polymeric material, a spindle, and two steel rails. One end of the spindle is coupled to a bearing attached to the fixed part and the other coupled

to the stepper motor that converts the rotational movement of the motor into a linear movement causing the mobile part to displace the syringe plunger (Figure 4).

Another part of the device is composed of two servo motors fixed in a rigid structure and coupled to two supports for connecting the "three ways" valves (Figure 5).

The device's electronic circuit consists of an Arduino Uno, an A4988 stepper motor driver, power supply, communication cable, and other electronic components (LEDs, resistors, buttons, among others). All components were installed on a test plate for assembling electronic circuits (protoboard) (Figure 6).

The power supply to the circuit (12VDC) and the communication cable is used as an interface between the computer and the Arduino to download the program. This cable can also be used as a power source for the Arduino. The servo motor and stepper motor cables are responsible for the communication between them and the electronic circuit board (Protoboard). The program that controls the Arduino terminals and actions can be developed using the software called Arduino IDE (available at https://.arduino.cc). The software is free and its programming language is wiring, derived from the C / C ++ language.

Device Commands

The commands inserted for the operation of the device were ON / START, STOP, and PAUSE. The ON / START button when pressed initiates the blood transfusion procedure. At any time during the experiment, if the STOP button is pressed, the blood in the syringe - from the RN or donor - is discarded and the device immediately stops. Also, at any time during the experiment, if the PAUSE button is pressed, the device stops and, if pressed again, the device returns to work from the point where it stopped. The PAUSE button can be used at any time if the user wants to pause the procedure to make any adjustments or any other action. STOP should be pressed in an emergency. A dropper sensor indicates when the donor bag is empty and the device automatically turns off, ending the blood transfusion procedure.

Experimental Procedure

The experimental procedure had the purpose of verifying the possibility to replace the technique of blood transfusion procedure by the exchange of the newborn's body blood volume for the volume of the blood component bag. The experiment took

Figure 4. The mechanical structure of the device.

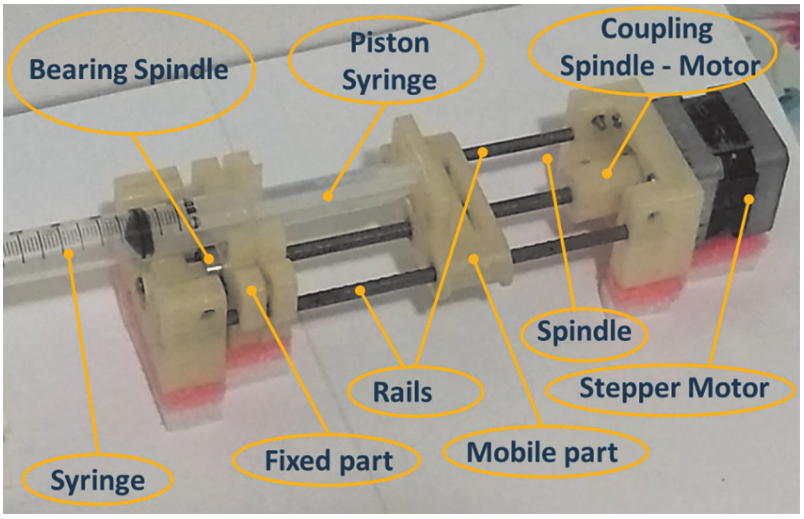


Figure 5. Rotating structure of the device.

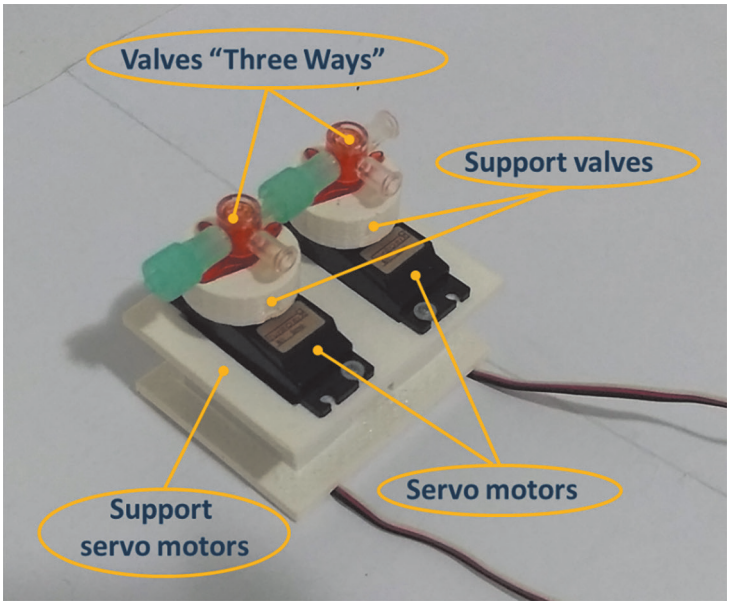
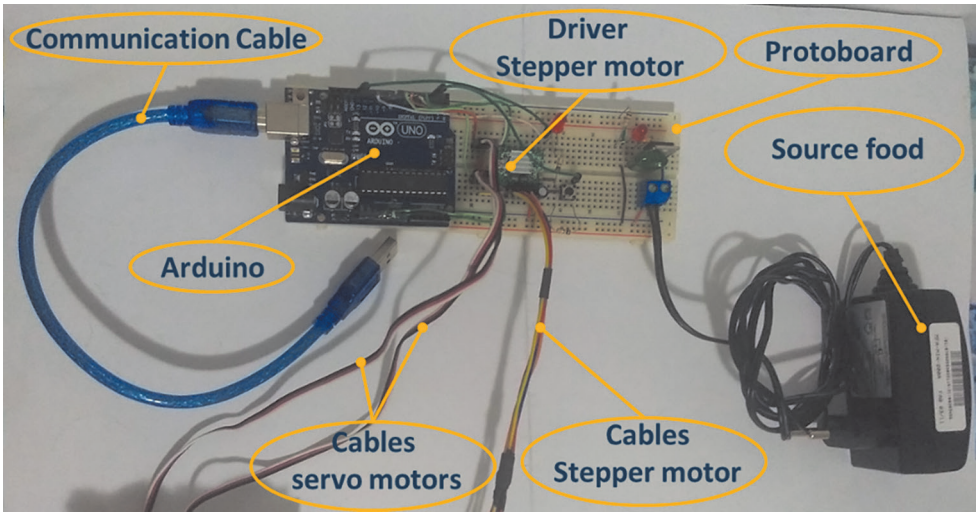


Figure 6. The electronic circuit of the device.



place on a test bench and the procedure time was divided into four cycles: a) time spent in aspirating the donor bag; b) time spent infusing blood in the newborn; c) time spent in aspirating the newborn's blood; d) time spent on blood disposal. It is worth mentioning that cycles "a" and "d" were programmed to be faster than cycles "b" and "c". The cycle "b" and "c" were directly related to the withdrawal and infusion in the newborn. The tests were carried out using yellow and red-pigmented water, in which the yellow color represented the blood of the newborn and the red color represented the blood of the donor.

Test Results

After carrying out the tests, the discard bags and the NB went orange, due to the mixture of yellow

and red pigments. Thus, it was demonstrated that there was a partial exchange of the NB's blood as expected, confirming the effectiveness of the automated device. The total time to perform the procedure, from the ON / START command until the end of the donor bag's content, was 35 min for a volume of 300 mL contained in the red bag representing the donor. The time was significantly shorter compared to the time of the procedure performed manually, which was achieved by adjusting the time in the stages of aspiration of the donor bag and blood disposal, preserving for safety the time in the phases of withdrawal and infusion as recommended by the Ministry of Health.

Results

This study resulted in automated device development, as an alternative flows in four cycles, with the potential to replace the manual procedure of blood transfusion therapy.

Discussion

This study presented a new device related to the procedure of blood transfusion in the therapeutic conduct of HDN. Currently, the manual procedure represents exhaustive, tense, and unsafe conduct by health professionals since it involves many sorts of situations such as calculating the volume of the infused blood component, the excessive volume of incorrect exchanges, the speed of infusion and blood withdrawal for disposal, control on the correct rotation of the valves, the records related to each movement performed adapted to the syringe, the possibility of the collapse of the umbilical vein at the time of blood aspiration, the possibility of pulmonary embolism related to the presence of air in the syringe during the procedure, care with handling the catheter in the umbilical stump and the possibility of infection caused by the invasive procedure. Many studies point out that the blood transfusion procedure is not without risks. Bradycardia, apnea, hypoglycemia, hypocalcemia, hyponatremia, and hypernatremia are mentioned as complications related to this volume replacement technique [20]. The most serious of the morbidities include symptomatic hypocalcemia, apnea, and bradycardia with cyanosis requiring resuscitation. These complications are common enough that the exchange transfusion, even in healthy newborns, should be performed only in Neonatal Intensive Care Units prepared to respond to these events [21].

At a time when many neonatal care providers have more experience with advanced therapies, such as high frequency of ventilation, dialysis, extracorporeal membrane oxygenation than with blood transfusion, a standardized protocol for performing the procedure can be an important tool to decrease the number of related adverse events [22].

Although an automated procedure does not eliminate the complications of blood transfusion and is likely to bring new inconveniences, it is believed that this device will bring greater safety to the team, freeing them from a tiring and repetitive routine, reducing the tension deposited during the manual procedure. So, the device can assume these actions providing more time in the humanized care to the newborn during and after the procedure such as the monitoring of the newborn's biochemical rates, heating of the crib, and use of post-procedure phototherapy.

Like other automated equipment, such as infusion by peristaltic pumps, safety mechanisms incorporated in the equipment guarantee total control of the volume to be infused per cycle, programmed time of infusion and withdrawal, flow control and speed of independent discharges, as well as a precaution against air inlet, infusion pressure sensor, and obstruction detector. Another proposal presented of the device is related to the reduction in the manipulation of venous access, decreasing the risk of possible infections. Also, the concern about the proper disposal of the blood drawn is a positive point of the device regarding the waste management plan in health services.

So, this functional device is an alternative infuser of four cycles and intends to carry out the automated procedure of blood transfusion.

Primarily there was the creation of a prototype, and in later studies, it will be tested in newborns proving its safety and effectiveness in specific hemotherapeutic treatments.

Conclusion

We concluded that it is possible to replace the manual and rudimentary procedures of the blood transfusion process with the development of an automated device, an alternative four-cycle infuser using three-way valves, Arduino logic, blood component bag, a disposal bag, and specific teams as integral parts of the process.

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Pancreatic Neuroendocrine Tumors: A Literature Review

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Pancreatic neuroendocrine tumors (PNETs) affect 1%-3% of patients with pancreatic cancer. This tumor is rare, difficult to diagnose, and clinically laborious. They have an estimated incidence of up to 1 case per 100,000 inhabitants every year. Up on diagnosis, most PNETs are considered malignant, with low healing potential, lesions that are generally unresectable, and a metastasis rate of aproximately 50%. PNETs are classified as functional and non-functional. The tumor functional produces hormones such as gastrin, insulin, somatostatin, glucagon, among others. They are symptomatic due to hormonal hypersecretion and occur in 30% of cases. The other 70% are non-functioning, and despite producing a series of substances and some hormones such as beta HCG and alpha HCG, they are silent tumors, with no significant clinical syndrome. The present study presented scientific evidence about PNETs, the types of pancreas endocrine-tissue tumors, the rate of survival, diagnosis, treatments, and prognosis, to provide solid support to professionals, and contribute to effective decision-making in search of the best clinical outcome.

Keywords: Neuroendocrine Tumor. Pancreatic Tumor. Functioning Pancreatic Tumor.

Abbreviations: NET: Neuroendocrine Tumor. PNET: Pancreatic Neuroendocrine Tumor. MEN-1: Multiple Endocrine Neoplasm type 1. VHL: Von Hippel-Lindau disease. NF-1: Neurofibromatosis type 1. F-PNET: Functioning Pancreatic Neuroendocrine Tumor. NF-PNET: Non-Functioning Pancreatic Neuroendocrine Tumor. WHO: World Health Organization. TNM: Classification System for Malignant Tumors. AJCC: American Joint Committee on Cancer. UICC: Union for International Cancer Control .

Introduction

Pancreatic neuroendocrine tumors (PNETs) affect 1%-3% of patients with pancreatic cancer. They are considered a rare tumor, difficult to diagnose, and clinical practice. The incidence is up to 1 case per 100,000 inhabitants every year [1-4]. Studied necropsies provide data that contribute to the prevalence of PNETs (0.5%-10%) [3,4]. Upon diagnosis, most PNETs are considered malignant, with low healing potential. The lesions are generally unresectable, and the metastasis rate is ≥50% [5]. PNETs are classified as functional and non-functional. Those that are functional produce hormones such as gastrin, insulin, somatostatin, glucagon, among others, and they are symptomatic due to hormonal hypersecretion. Their prevalence is 30% of the PNETs' cases. The non-functional tumors (70% of the PNETs' cases) are silent with no significant clinical symptoms, despite the production of substances and some hormones such as beta and alpha HCG [2,3].

Koo and colleagues [6] showed that metastasis of PNETs can be discovered before primary tumors. The proposal of the study was based on the use of PABX8 markers and Islets 1, which was used for immunohistochemical tests of pancreatic origin's NETs. The study included 110 samples of primary PNETs and 73 of NETs, of which 28 were pancreatic, 5 pulmonary, 37 ileum, 1 rectal, 1 colon, and 1 duodenal. The results showed that Islets 1 had 68% of specificity for metastatic PNET, revealing that it can be used for investigation of this tumor; and PABX8 remains more specific as a primary PNET marker, with 88% of primary PNET samples detected by this marker [6].

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PNETs are uncommon and without a germline-associated mutation. KRAS gene mutations, commonly found (somatic) in pancreatic adenocarcinoma, are almost completely absent in pancreatic NETs. Genetic syndromes such as multiple endocrine neoplasia type 1 (MEN-1), Von Hippel-Lindau disease (VHL), neurofibromatosis type 1 (NF-1), Mahvash's disease, and tuberous sclerosis (TS) have been correlated with the development of NETs in several studies. Around 12% of patients with VHL develop PNET, while NF-1 and TS have been reported casually. However, there isn't much data due to their rareness. On the other hand, most patients with MEN-1 have PNET, and typically numerous microadenomas, which may be associated with the premature death of these patients [7].

So, this study presents scientific evidence about PNETs (the types of pancreas endocrine-tissue tumors, the rate of survival, diagnosis, treatments, and prognosis) to provide solid support to professionals and contribute to effective decision-making in search of the best clinical outcome.

Methods

We carried out a literature review in the academic databases such as Pubmed and Scielo, using the terms "Neuroendocrine Tumor", "Pancreatic Tumor" and "Functioning Pancreatic Tumor", and new technology platforms that select the most cited articles crossing them with high impact journals (> 7.0). However, for some references, we used only the most cited articles. We used reviews and cases' studies about the theme, diagnosis, treatment, and survival of patients with Pancreatic Neuroendocrine Tumors. We utilized a few articles published 12 years ago, and we also researched the World Health Organization classifications for NET of pancreatic origin.

Functioning Pancreatic Neuroendocrine Tumors (F-PNETs)

The functioning PNETs are producers of hormones such as gastrin, insulin, somatostatin, glucagon, among others, and are symptomatic due to hormonal hypersecretion. In order of frequency, PNETs are Insulinomas (Beta cells), Gastrinomas and Glucagonomas (Alpha cells), Somatostinomas (Delta cells), Vipomas (Delta 2 cells) e PPomas (PP cells). Since 2010, according to the World Health Organization (WHO), PNETs should be classified based on cell proliferation and tumor morphology. One of the markers of cell proliferation rate is Ki-67 [2,3,8]. WHO also classifies PNETs as 1) well-differentiated, less aggressive; and 2) poorly differentiated, highly aggressive [2]. When compared to pancreatic adenocarcinomas, whose survival is 5-year (~6%, varying from 2%-9%), PNETs are slow-growing and have a better prognosis [3]. In a retrospective study by Sanchez-Bueno and colleagues [4], in which medical records of 95 patients after resection of TNEPs were reviewed, the 5-year survival rate was 100% for well-differentiated tumors. They considered variables such as age, sex, classification of tumors as sporadic or familial, functioning or non-functioning tumor, type of tumor, location, surgical technique, tumor size, multifocal tumors, and recurrence rate [4]. Concerning the diagnosis of PNETs, positron-emitting tomography integrated with computed tomography (PET/CT) associated with radiopharmaceuticals is a promising technique in recent years [9].

Non-Functioning Pancreatic Neuroendocrine Tumors (NF-PNETs)

NF-PNETs are originated in pancreatic tissues and do not produce any substance capable of causing clinical syndromes. These tumors secrete chromogranin A and B (90%-100%), Alpha-HCG (Human Chorionic Gonadotropin) (40%), Neuron Specific Enolase (31%), as well as Beta-HCG (20%). As 40%-90% of NF-PNETs secrete pancreatic peptides called PP, they are also called PPomas [8].

NF-PNETs can additionally secrete ghrelin, neurotensin, calcitonin, and other neurotransmitters.

This is a silent tumor in which 30%-40% continue to behave with nonspecific symptoms, only being detected after screening for many symptoms, typical of other diseases and syndromes. The manifestations of NF-PNETs are produced internally by the tumor, due to the tumor mass. So patients end up looking for help late, and 64%-92% of them already have liver metastasis, and 72% with tumors >5cm. These tumors are commonly solitary, with a small exception of patients with MEN 1, in which the manifestations occur in multiple tumors, mainly in the head of the pancreas. Most NF-PNETs, when compared to F-PNETs, can be considered asymptomatic, since they do not present significant symptoms to characterize the clinical condition of the disease and cannot be differentiated from the functioning pancreatic endocrine tumors by immunohistochemical. Studies show that 80%-100% of patients with MEN-1 have microscopic NF-PNETs, and these tumors become large and symptomatic in the minority of cases (0-13%). In VHL disease, 12%-17% of patients develop NF-PNETs, and 4% of them reach ≥3 cm. The symptoms commonly presented by patients are abdominal pain (30%-80%), jaundice (20%-35%), weight loss, fatigue, and bleeding (low proportion) [8].

Classification of Neuroendocrine Pancreatic Tumors

The prognosis of PNETs can be established according to size, histological findings, and staging. Since 2010, the classification by World Health Organization (WHO) divides PNETs into three degrees (G1, G2, and G3) based on expression Ki-67 of the nuclear antigen (<2%, 2-20%, and >20%) and on the rate of mitotic division (<2%, 2%-20% and >20%): G1 and G2 are referred as NETs and G3 as neuroendocrine carcinomas. NETs well and moderately-differentiated (G1/G2) show a higher survival rate when compared to poorly-differentiated neuroendocrine carcinomas (G3). According to the Classification of Malignant Tumors (American Joint Committee on Cancer and Union for International Cancer Control - AJCC/UICC), the tumor is classified into T1a (<1cm), T1b (1-2cm) and T2 (>2cm); T3 and T4 are locally advanced tumors (Table 1) [10].

Histologically, NETs are classified into typical and atypical (anaplastic). Typical tumors are well-differentiated, composed of a small cell mass, with regular cells and rounded nucleus. Atypical tumors have nuclear atypia and accelerated mitotic activity concerning typical tumors, as well as areas of necrosis. There are five distinct histological patterns: insular, glandular, undifferentiated, differentiated, and mixed (Table 2). Nevertheless, we emphasize that there are exceptions in cellular behavior based on histological characteristics, but it should be considered that some histological characteristics determine NET tumor behavior, such as vascular, lymphatic, and organ wall invasion, degree of cellular atypia, nucleocytoplasmic ratio, presence and extension of tumor necrosis and mitotic index. Markers of cell proliferation (Ki-67 Index) and p53 expression are important in determining tumor aggressiveness [11].

Insulinomas

Insulinomas are tumors characterized by hyperfunction of beta cells that produce insulin in the pancreatic islets. These tumors are intrapancreatic, and extraglandular involvement is rare. Considered the most common PNETs, they correspond to 35%-40% of the endocrine pancreas' lesions [7, 9, 12]. It is a benign tumor in most cases (85%-99%), single (93%-98%), <25mm in diameter. However, 5%-12% of insulinomas are malignant and >30mm, and up to one-third of malignant insulinomas have metastases at the time of diagnosis. In cases with multiple tumors (10%) half of them are associated with MEN-1 [9, 13]. Insulomonas have a high frequency in females (59%), they can appear at any age, but they are predominant from 45 to 50 years, except for patients with MEN-1, in which they are commonly diagnosed at 25 years of age. The possibility of diagnosing MEN-1 should be investigated in all

Table 1. Neuroendocrine tumors classification.

NET Classification	Ki-67 index
Well-differentiated neuroendocrine tumor (also called typical carcinoid)	<2%
Well-differentiated neuroendocrine carcinoma	2%-15%
Poorly-differentiated neuroendocrine carcinoma	

Source: Adapted from Araújo and colleagues [11].

Table 2. Classification AJCC/UICC.

NET	AJCC/UICC
T1	Confined to the pancreas, <2cm
T2	Confined to the pancreas, >2cm
T3	Peripancreatic spread, but without large vascular invasion (upper celiac or mesenteric vessels)
T4	Major vascular invasion (celiac axis or superior mesenteric artery)

Source: Adapted from Braga and colleagues [5].
Source: Adapted from reference Araújo and colleagues [11]

patients with insulinoma [9, 12]. This pancreatic tumor is the most common cause of hypoglycemia - relating to endogenous hyperinsulinism - and the incidence of insulinomas is 1-4 cases per 1 million people [14]. Insulinomas are clinically manifested by the Whipple triad: (1) episodic hypoglycemia with low glycemic values (40-50mg/dL) and (2) central nervous system (CNS) dysfunction associated with hypoglycemia (this manifestation may progress to mental confusion, anxiety, stupor, and coma), and (3) such dysfunction can be reversed with the administration of glucose. Caldas and colleagues [9] showed a case study that in addition to the hypoglycemia associated with the Whipple triad, 29% of the patients exhibited postprandial hypoglycemia, 21% fasting, and postprandial hypoglycemia, and only 6% manifested just hypoglycemia. This same study recommends that the diagnosis of insulinoma should consider the patient's clinical history, and not be excluded based only on the moments when hypoglycemia occurred, since the average between the onset of symptoms and the diagnosis is 19-20.9 months [12]. Common autonomic symptoms of insulinoma are diaphoresis, tremor, and palpitations, while neuroglycopenic symptoms include, confusion, behavioral changes, personality changes, visual disturbances, seizures, and coma [7, 14].

As insulinoma patients could have neuropsychiatric symptoms, it is common a diagnostic error (approximately 20% of cases). This reveals why is important the knowledge of the patient's clinical history avoiding late diagnosis of the disease. In some cases, due to an absence of adrenergic symptoms associated with acute hypoglycemia (confusion, seizures, and coma), the patient with this condition, which is still reversible, ends up in other medical areas not related to the real condition, which compromise the treatment and the best clinical outcome [9, 12]. So, the diagnosis of insulinomas is based on the clinical history of patients, laboratory confirmation, which implies the demonstration of high levels of insulin (40-55UI/mL), spontaneously or caused by prolonged fasting tests. Approximately 97% of insulinomas are diagnosed before 45 hours of fasting, despite the protocol following 72 hours. Elevated levels of C peptide are also considered (average of 4.5ng/mL and a maximum of 8.4ng/mL), which allows the exclusion of false hypoglycemia. Imaging tests should be performed after biochemical confirmation, due

to 80% of insulinomas have a diameter <2cm, which is difficult to be located. The search for the preoperative location should be performed exhaustively before surgical resection to reduce the manipulation of the pancreas during surgery since 10%-27% of insulinomas not preoperatively located are also not located intraoperatively [9, 12, 15]. Echoendoscopy is the most successful method in cases of preoperative localization, associated with computed tomography (CT) with multidetector and intravenous contrast, in which sensitivity can reach up to 100%. However, due to failures in locating insulinomas, other methods for diagnosis are used and improved in studies, such as ultrasound with contrast or scintigraphy marked with peptide 1 Glucagon-like, which has been showing promising data [13].

Surgery is the main treatment for insulinomas, but after resection, the major patients need symptom control and long-term cure. For well-differentiated primary and metastatic functional PNETs, surgery should always be considered, even when the liver or regional lymph nodes are involved. In the case of unresectable metastatic disease, despite the controversies about the morbidities associated with surgery, such as pancreatic fistula, postoperative hyperglycemia, surgical site infection, late diabetes, among others, the resection of the primary lesion is considered palliative but appropriate to symptomatic control and a better outcome. Studies have suggested improvement of overall long-term survival and reduction of disease progression after resection of the primary tumor in the advanced stage [10, 16]. Surgeons describe several types of surgical approaches; and the most cited is insulinoma enucleation and partial distal pancreatectomy. Currently, laparoscopic resection of insulinoma is frequent due to shorter hospital stay and beneficial results to other similar procedures. Robotic enucleation of insulinoma, although little known, presents good results in the short and long term when compared to the laparoscopic approach [16]. Physicians may try pharmacological treatment before surgery in recurrent cases or for malignant insulinomas. For early insulinomas, it is recommended that changes in diet, treatment with medications such as diazoxide or everolimus, and the use of medical bracelets are prudent steps. Therapeutic alternatives, including glucagon pen, somatostatin analogs, and steroids, are also considered [17]. Somatostatin, produced in pancreatic delta cells, acts via paracrine regulation of insulin and glucagon secretion, also regulating cell proliferation, through interaction with five different types of somatostatin receptors coupled to G protein, called SSTR1-5. These SSTR subtypes have distinct molecular structures,

Table 3. Procedures for locating insulinomas.

Procedure	Sensitivity
Abdominal Computed Tomography	70%-80%
Preoperative Abdominal Ultrasonography (US)	13%-67%
Abdominal Magnetic Resonance	Up to 85%
111In-pentreteotide Scintigraphy	50%-60%
Dosage of Insulin in the Hepatic Vein after Intra-Arterial Infusion of Calcium Gluconate	91%-100%
Pancreatic Endoscopic Ultrasonography	60%-90%
Transoperative Pancreatic Ultrasonography	91%
Transoperative Pancreatic Palpation	75%-90%
US + Transoperative Pancreatic Palpation	~100%

Source: Adapted from Santos [15].

tissue distribution, intracellular signaling, and pharmacological characteristics that inhibit different types of hormones. SSTR2 is the dominant receptor on alpha (glucagon) and beta (insulin) cells [10].

Diazoxide is the appropriate option if the patient has an unresectable tumor, metastatic disease, or if it is a patient to which surgical treatment is not recommended. A significant reduction in the release of insulin and an increase in glycogenolysis is expected with the use of diazoxide. A rate of 60% of the patients achieves the expected results with symptoms-free. The efficacy of octreotide in patients with refractory hypoglycemia is smaller, but it remains an option for patients who do not respond to diazoxide. For patients with refractory symptoms and stable tumor volume, adjustment of the dose of octreotide, a potent inhibitor of somatostatin, GH, glucagon, and insulin, as well as surgical resection, may be considered [9]. Considering that most of these tumors are of benign origin, surgical resection (laparoscopic or laparotomy) is the treatment of choice with a high rate of cure (75-98%). However, the prognosis depends on the stage of the disease and/or if the resection is achieved completely [18].

Gastrinomas

Gastrinomas, also called Zollinher-Ellison Syndrome (ZES), come from G cells, present in the duodenum, pancreas, and stomach. These tumors ectopically secrete gastrin, causing hypergastrinemia and, consequently, an increase in the secretion of gastric acid, which results in peptic ulcers. Some pathologists classify as gastrinomas some tumors that present gastrin in assays by immunocytochemistry (such as bronchogenic carcinomas, colorectal cancer, among others). However, these tumors, although containing gastrin, are not associated with hypergastrinemia and cannot be recognized as gastrinomas or ZES [19].

The incidence of gastrinomas is 0.5-1.5 cases per 1 million people per year. They are tumors located in the duodenum (70%) or pancreas (20%-25%). Approximately 80% are sporadic and 20%-25% are associated with MEN-1. More than half of gastrinomas are malignant and have a high potential for infiltrating regional lymph nodes and liver metastases [19, 20].

Serum gastrin levels ten times above the normal limit (>1,000pg/mL), in the presence of gastric secretion with a pH below 2, is suggestive for the diagnosis of gastrinoma. When there are moderate changes in baseline gastrin levels, a secretin test should be performed. For example, an increase in gastrin levels above 120pg/mL concerning baseline fasting levels suggests investigation for gastrinoma, with sensitivity and specificity of 94% and 100%, respectively. Gastrin levels should be measured after discontinuing treatment with PPI proton pump inhibitors (omeprazole, pantoprazole) for at least 5 to 7 days, considering that PPI-induced hypochloridia is one of the most frequent causes of hypergastrinemia. However, in patients with ZES, discontinuation of PPI can cause an abrupt and dangerous recovery of acid secretion, therefore, some authors recommend diagnostic evaluation under the protection of PPI [20].

For diagnostic confirmation, numerous methods of tumor localization are performed, including computed tomography (CT), ultrasound, selective angiography, magnetic resonance, a functional location that measures hormonal gradients, endoscopic ultrasound, and scintigraphy of somatostatin receptors (SRS) using 111 In-DTPA-DPhe-1-octreotide with a 73% detection rate for gastrinomas. Recent studies demonstrate that SRS is presently the modality of choice for the diagnosis of both localized and metastatic disease [21]. The initial treatment consists of a drug approach with histamine H2 blockers or PPI proton pump inhibitors, followed by an imaging exam for a surgical approach. It is impossible to be cured without surgical procedures with radical resection in patients with MEN-1-associated gastrinoma, due to multiple and small tumors and the tendency to metastasis. However, in MEN-1 patients the

surgical indication remains controversial due to short and long-term complications, which is why a Total Pancreatectomy (TP) is rarely performed [19].

Before the use of H2 antagonists and PPIs, the surgical procedure sought primarily to control symptoms and prevent sequelae. The surgical approach mainly involved total gastrectomy, vagotomy, or, if it is possible, the resection of the tumor mass. Drug therapy in the treatment of gastrinomas provided adequate management of the patient's clinical condition, changing the role of cancer surgery for localization and resection of the primary and metastatic tumor. In the case of sporadic tumors, the surgical approach is indicated as curative, unless there are other contraindications. Surgical exploration is performed through laparotomy for pancreatic gastrinomas and duodenopancreatectomia for duodenal gastrinomas, in addition to the dissection of regional lymph nodes, if there is involvement. Tumors of the pancreatic head should preferably be enclosed and lesions on the pancreatic body or tail require intermediate or distal pancreatectomy. The Whipple procedure (PD) is restricted to exceptional cases [5, 20].

Despite high cure rates, biochemical and morphological recurrences are frequent during follow-up. The improvement in survival due to surgery has only been demonstrated recently because these tumors usually progress very slowly and a long follow-up is required. Survival for patients with gastrinoma and liver metastasis is 20%-30% in 5 years [20].

Glucagonomas

Glucagonomas are PNETs that originated from the alpha cells of pancreatic islets. Considered as a rare tumor, the approximate incidence is 2.4 cases per 100,000,000 people in America, and only 400 cases have been described in the literature. According to the classification of tumors of the digestive system of the World Health Organization (WHO), Glucagonomas are functioning PNETs, and manifest with typical multiple symptoms, such as weight loss of 10 to 15kg, diarrhea, diabetes mellitus, venous thromboembolism, migratory necrolytic erythema, rash, steatorrhea, anemia, depression, and neuropsychiatric factors. However, early diagnosis, which could provide curative resection, remains difficult due to the uncommonness of this disease [22, 23, 25]. Glucagonomas similarly affect men and women and are usually large tumors (>4cm), whose anatomical site is frequent in the distal part of the organ (body and pancreatic tail). They are commonly diagnosed from ages 40 to 50 years, and 50% of patients present metastases at the time of diagnosis [22, 23, 26].

Changes in plasma glucagon levels may be associated with the appearance of Glucagonomas, so this may be one of the criteria for diagnosis. Even so, caution is necessary, as slight changes in serum dosage above 150pg/mL are also present in other clinical conditions, such as difficulties in absorption, infection, and liver cirrhosis. Studies have shown that mutations in the gene responsible for the glucagon receptor are also associated with elevated serum levels of this hormone and that the use of some drugs such as estrogen replenishers in patients with Protein S deficiency has also been correlated with high levels of glucagon [25, 26]. Despite this, fasting levels above 500pg/mL can be considered a warning sign (normal range is 50-150pg/mL) since the diagnosis of glucagonoma requires an elevated serum level of glucagon (500-1000 pg/mL). However, considering the possibility of other conditions present high levels of glucagon, imaging methods are important to confirm the pancreatic tumor [23, 24, 26].

The location of the tumor is performed by computed tomography. Pancreatic islet tumors and metastases are usually hypervascular with better definition during the arterial phase of contrast. Confirmation and staging of the tumor can be performed in all patients by SPECT/CT OctreoScan with 111In-pentreteotide (scintigraphy with somatostatin receptors - SRS), and more recently with Ga-DotataTE PET/CT.

Another useful of scintigraphy is to evaluate the functional status of the tumor and the secondary location, providing appropriate therapy with peptide receptor radionuclide (PRRT). Treatment may vary depending on the stage of the disease, but currently, surgical resection is the only one that presents a cure chance for glucagonoma (NME can disappear within 1 week after surgery) [22, 25]. Somatostatin analogs and amino acid solution infusion result just in symptom relief. Other therapies considered useful are transarterial chemoembolization, radiation therapy, and peptide receptor radioligand therapy. Patients may feel significant improvement after treatment and cognitive improvement after surgery, suggesting that neuropsychiatric symptoms are due to the high level of glucagon [25,26]. Despite the malignancy of the disease, overall survival even in patients with metastasis ranges from 3 to 8 years [22]. A patient with glucagonoma may have Necrotic Migratory Erythema (NME), characteristic of excess glucagon serum. Glucagonoma syndrome, when not considered therapeutically as a pseudoglucagonoma syndrome, maybe a tumor in the alpha cells of the islets of Langerhans, and the investigation for glucagonoma is recommended when the patient has NME [23, 24, 26]. The mechanisms of glucagonoma-related changes and disorders have yet to be elucidated, but studies show that 76-94% of patients with glucagonoma can develop diabetes mellitus and 20% of patients have neurological and/or psychiatric disorders such as dementia, psychosis, agitation, paranoid delusions, ataxia, and hyperreflexia [23, 24, 26].

Somatostatinomas

Somatostatinomas are rare tumors with an incidence of 1 case per 40 million people. These tumors originate from the D cells of the apud system (endocrine cells) can develop in the tissues of the pancreas and duodenum. About 56%-70% of somatostatinomas are from pancreatic tissues, 36% in the head of the organ, 14% in the body, and 32% in the pancreatic tail [27]. Somatostatinomas can be functional or non-functional NETs. Duodenal somatostatinomas are commonly non-functional and pancreatic ones are often secretory, also known as functional tumors, in which clinical effects are linked to somatostatin secretion. Somatostatinomas are often malignant (90%), and approximately 30% of the duodenal ones and 70%-88% of the pancreatic ones present metastases at the time of diagnosis. Around 40% of the metastases are hepatic and 30% in the lymph nodes. Pancreatic somatostatinomas are most often larger than duodenal ones, with an average size ranging between 0.3 and 6cm [27, 30]. The duodenal somatostatinomas are commonly associated with nonspecific symptoms, with neurofibromatosis and less frequently with somatostatinoma syndrome or metastasis [27]. Symptoms are not typical and can be confounded with other diseases such as diabetes, indigestion, and cholelithiasis since when the tumor is secretory, patients have somatostatinoma syndrome, characterized by diabetes, gallstones, and steatorrhea, which are similar to other diseases. The diagnosis is confirmed by histological and immunohistochemical studies and by the presence of specific hormones [30]. If the tumor has a duodenal origin, the diagnosis of somatostatinomas can be performed through upper digestive endoscopy followed by a biopsy, anatomopathological, and immunohistochemical examination. For pancreatic somatostatinomas, the most used methods of diagnosis are computed tomography, ultrasound (US), and nuclear magnetic resonance associated with increased serum somatostatin levels (>2.5pg/mL in 80% of cases), and serum levels of Chromogranin A and Pancreatic Polypeptide used as nonspecific markers of PNETs (50%-80% of cases). The endoscopic US can be used to know the extension of the lesion and the locoregional lymph nodes involvement. The somatostatinomas are well located and present in solitary nodules with an average diameter of 5cm. In general, diagnosis is confirmed through anatomopathological and immunohistochemical examination, with the use

of chromogranin A, neuron-specific enolase, and synaptophysin as immunohistochemical markers. Approximately 65% of cases allow for complete resection [29, 30]. Since most somatostatinomas are located in the periampullary duodenum or the head of the pancreas, a partial pancreatoduodenectomy that preserves the pylorus is the resection performed in patients with somatostatinoma, although total pancreatoduodenectomy may be necessary. As a high proportion of patients have metastases, the role of clearance must be carefully analyzed [30]. Despite surgical represents the only option with curative chances, palliative patients have benefits from surgery for pain relief, clearance of the biliary and intestinal tract, and control of symptoms associated with high serum levels of somatostatin in functional tumors. Chemotherapy is a treatment option for symptom control, trying therapeutic benefits in cases where curative or palliative surgery is not possible [29].

Somatostatin analogs are used to relieve hormonal symptoms, but in severe disease, therapeutic options are limited. Results obtained by Noda and colleagues [4] reveal that the gamma subunit of the high conductance calcium channels (BKCa2) (expressed in many tumor-cells such as ovarian tumors, osteosarcomas, breast tumors, and gliomas), whose function is to modulate proliferation, migration, and metastasis, are strongly involved in the proliferation of human somatostatinoma cells, which can suggest a new therapeutic target [4, 28]. The five-year survival rate for patients with pancreatic and periampullary somatostatinomas is 60%-100% in cases of localized disease, and 15%-60% with metastatic disease. When total resection is achieved, in both cases the five-year survival can reach 100%. Extensive (>3cm), poorly differentiated tumors, and lymph node involvement are poor prognostic markers. In 75% of cases, tumors >3cm have hidden hepatic metastasis, contributing to the poor prognosis. Non-functional and poorly differentiated tumors have a worse prognosis than functional somatostatinomas [29, 30].

Final Considerations

In this article, we demonstrate the difficulty in the management of patients with pancreatic NETs, due to the late diagnosis, which contributes to low patient survival and impaired quality of life. However, it is possible to achieve significant gains based on the data and improvement of progression-free survival in some cases. The slow development of these tumors and an early diagnosis is possible through careful investigation of patients' clinical history, family history, the use of biochemical exams such as serum levels associated with symptoms, and advanced imaging methods, as well as immunohistochemical analysis and exploratory surgery. Also, the presence of other diseases and comorbidities related to pancreatic NETs should be done.

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Internet Access in the New Brazilian Normal: A Basic Need for Access to Income, Health, and Education

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Remote access to information and services has become a latent reality in Brazil since the emergence of the COVID-19 pandemic in 2020. This paper discusses the importance of the community's access to the internet, and as the web's worldwide is an essential tool for remote working and a supporting pillar for essential services remotely offered such as education, banking, and medical care.

Keywords: COVID-19. Teleworking. Telemedicine. Remote Education. Banking Platform.

Introduction

In 2020, the world faces one of the biggest global public health crises due to the impacts of the new coronavirus (COVID-19), which started in December 2019, in the Wuhan region, China [1]. Social isolation has been adopted as one of the main recommendations from the World Health Organization, aiming to mitigate the impacts of the pandemic, and also to guarantee medical care due to the number of ill people. Controlling the contagion curve of the virus until a treatment or an effective vaccine against COVID-19 gets available is a key objective of the global community.

In this context, areas of the economic sector that have a direct impact on society, such as health, education, and financial aid providers, should be the first to benefit from a flexible working model. Major internet facilities and access then turn to be key enablers to keep essential services available to the community.

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Teleworking, Sustainable Development, and Corporate Development

In the last decades, there was an important growth in both the distance and the time spent on journeys to work. Nowadays around the world, disregarding some metropolis that has a good subway infrastructure, this journey, or part of it, is performed by cars, motorcycles, or buses [2]. In this way, these people's workaday journey is responsible for about 12% of Carbon Dioxide 2019 emissions globally [3]. The World Greenhouse Gas Emissions detailed the distribution of Carbon Dioxide in 2016 around the world (Figure 1) [4].

With the COVID-19 pandemic, many companies had to incorporate the teleworking or home-office model, aiming to follow with your normal operations working. However, it speeds up a work model flexibilization that had being slowly growing. Thus, in some months is already possible to perform a pre-analysis of many subjects related to teleworking, whether positives or negatives (Figure 2) [2].

Considering all the global engagement with a focus on sustainable development that preserves intergeneration equity, and by contrast, the constant need for companies' productivity improvement, two main teleworking advantages could be highlighted: Employees productivity growth due to daily time gain and worktime flexibilization, resulting in better life quality. Polluting Gase's decrease due to the lower road transport demand on work trips.

Figure 1. World Greenhouse Gas Emissions in 2016.

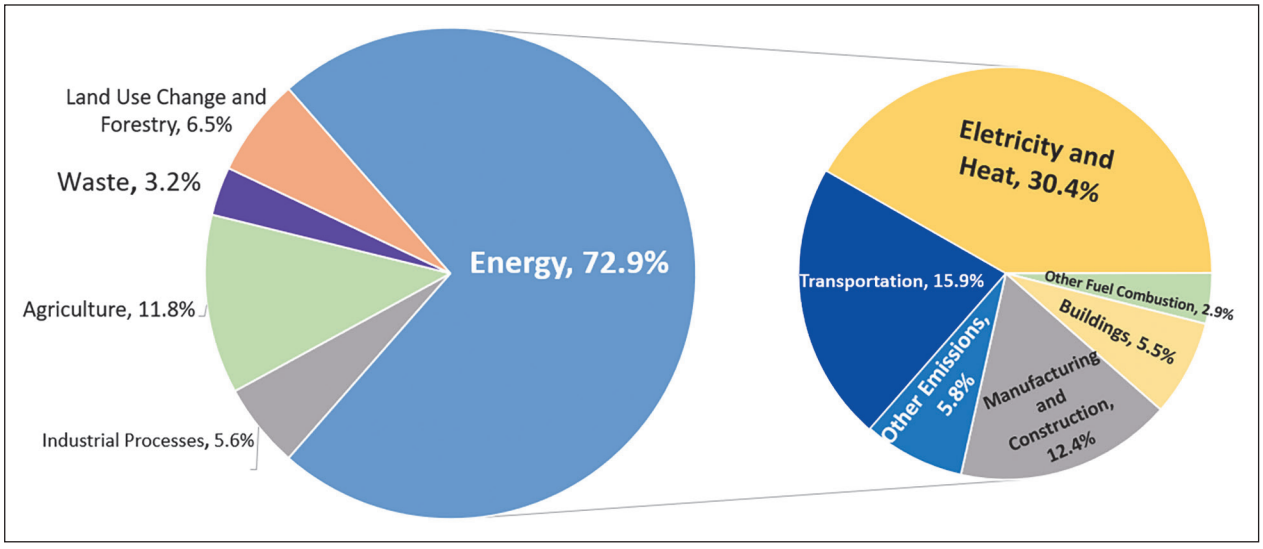
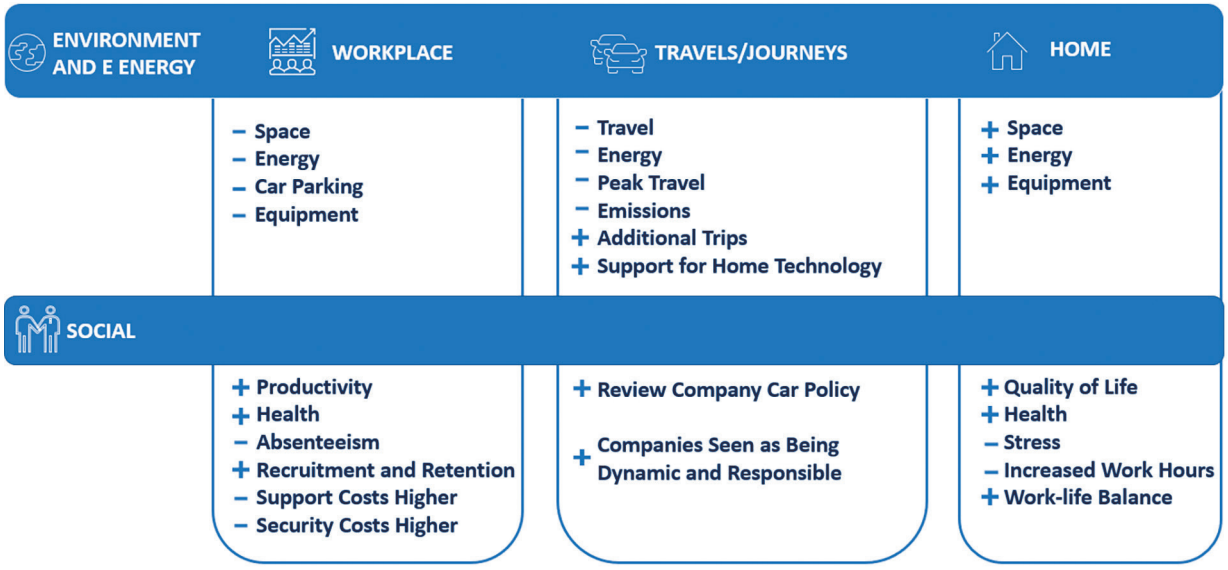


Figure 2. Home-Office advantages and disadvantages.



Access to COVID-19 Emergency Financial Aid: The Social Invisibles Citizens

The social isolation policy during the 2020 COVID-19 pandemic period may be more restrictive depending on the economic and public health situation in each country, but, in general, it directly affects the industrial and commercial activities considered as non-essential. In other words, those activities that are not linked to the guarantee of health, food, and supply of the necessary goods to maintain the productive chain available for public services and essential activities were not allowed to keep working.

The halt of several economic sectors due to Brazilian social isolation decrees had serious impacts reducing the already deteriorated family's incomes [5]. Mostly affected families are exactly the ones that do not enjoy the privileged of public sector job stability or do not have earnings

beyond the necessary to cover their basic needs. These families in general have a higher social vulnerability in which people are linked to informal variable income jobs and, consequently, are excluded from constitutional guarantees such as a Time Service Guarantee Fund (FGTS), Social Integration Program (PIS), and unemployment insurance. Furthermore, they do not have the support of unions either homologation of formal maintenance job agreements considering salary reductions. Aiming at this public of lower-income, an emergency aid of R$ 600 was created by Federal Government, initially in force for a period of 3 months.

The absence of a single national registry repository that makes it possible to identify all people able to receive the government's financial aid generated the need for a new registry for those who claim access to assistance. Since the decision that emergency aid would be paid, many efforts have been made to keep people at home to maintain social isolation. In this sense, the applicant's registration for assistance started to be made preferably via Caixa Econômica Federal cellphone application or webpage which requires plaintiffs' access to the internet. Some state institutions and banks' inefficiencies were revealed to the public during this period. The relevant state's challenge in analyzing social and monetary needs from the population would require an intelligent, robust and centralized government data system, robust enough to avoid delays on resource releases for those who need it most, and also avoid system's frauds.

According to data released in the National Household Sample Survey [6] containing data collected in a survey conducted between 2017 and 2018, about 21% of Brazilians do not have access to the internet at home. The main reasons for not using the internet at home pointed out by the research were: lack of interest in accessing the internet (34.7%), internet access service was too expensive (25.4%) and no resident knew how to use the internet (24.3%). Also, internet access in Brazil is unequal and has different access levels depending on several factors such as housing area (urban or rural), the region of the country, age group, and per capita income. A certain pattern was identified as common in the survey: the household average real income per capita where there was internet frequent usage (R$ 1,769) was almost double compared to the income of those who did not use this network (R$ 940). The great difference between these two income groups was observed in all the Major Regions [6]. It is evidenced through the research that those who most need emergency assistance are the people who have restricted access to the internet

Telemedicine

Telemedicine started to be used in Brazil in the earlier 90s, in health teaching and research establishments, having its prelude with the Disque Saúde in São Paulo, established as a pioneer information service in 1989, becoming an appointment consultation service in 1991, and expanded to other regions such as Contagem (Minas Gerais), Vitória (Espirito Santo), Curitiba (Paraná), among others [7]. Disque Saúde was an enabler for subsequent programs like Rede Universitária de Telemedicina (RUTE) and the program Telessaúde Brasil, with specific activities in the SUS network, paving the way for future regulations on teleconsultation.

Even considering its long existence, there is still a great dependence on the success of the grouping of existing technologies, to build and allow their integration, supporting complete solutions for the application of telemedicine. Investments in infrastructure, information standards, and systems applied to the area will assure interoperability, services, and a management model, not only limited to financial resources, but also for their development and constitution. Generating as consequence barriers that allow doctors to use their techniques efficiently on remote patient care, accentuated by the moment of the COVID19 pandemic, such as licensing providers for performance and payment; medical malpractice insurance for telemedicine; adherence

to confidentiality and security regulations; establishment of protocols to manage laboratory tests, prescriptions and programming.

The adoption of telemedicine, within this context and as one of the arms of telehealth actions, according to Caetano [7], can bring benefits, such as the reduction of time of attendance, the costs of transportation for patients and health professionals, improving quality assistance, in addition to allowing specialists to access remote areas, previously not covered by ordinary and face-to-face routes.

To improve the digital health area in Brazil, the government created the Department of Digital Health in 2019, linked to the Ministry of Health, which has implemented National Digital Health Policy and Telehealth in SUS. It covers the entire spectrum of formulation, planning, coordination, monitoring, and evaluation of this implementation, in addition to the development of processes for the elaboration, negotiation, implementation of standards and instruments necessary for the practice of digital health in SUS. It also promotes the expansion of the information network, communication, and integration at the national level, in addition to stimulating and encouraging the exchange of knowledge and experiences between public and private entities, the scientific-technical community, and international organizations working in the field of telehealth, telemedicine and digital health [8].

It is worth mentioning that the successful implementation of telemedicine in the country within SUS, the government, through the Department of Digital Health, should rely on formal and informal links between companies, research institutes, universities, and the government itself, using the networking already established to leverage effective implementation in SUS and country coverage, while promoting the grouping of enabling technologies, human capital formation, and research to improve existing services, in addition to a structured approach to the challenges for successful dissemination of these in the national territory.

Remote Education

According to Castaman [9], remote education, known as RE, is experiencing an acceleration process due to the context of the COVID19 pandemic. Countries such as Brazil, China, and the United States are developing significant changes associated with remote learning, despite being at different levels of adherence to this methodology. History, features, and opportunities related to RE will be addressed in the next paragraphs. Zhou [10] states that in China, remote learning initiatives started in 1978 and showed great acceleration since 2012, with 70.2% of Chinese schools with full multimedia capability. Strengthening its policies in the pandemic period, the country launched an online national education program known as "School's Out, but Class's On". The great challenges in its implementation were an adaptation to the remote teaching method and the lack of student's self-autonomy at home. It is a country with 270 million students in basic and secondary education, and 20 million university students, increasing the complexity of initiatives. In Brazil, according to Castaman [9], the strategy related to remote learning started in 1996 through the Law of Guidelines and Bases, being accelerated only in 2015 by the National Education Plan. Provisional Measure 934, from 2020, interrupted face-to-face classes, recommending substitution by remote learning during the pandemic period. Through a survey of 3,300 respondents, the National Emergency Committee Department found that approximately 50% of students face learning difficulties in remote activities. Alternatives to the maintenance of learning in the country become essential in face of such scenario. Bailey [11] states that strategically addressing distance learning has positive results. In the work, the author elaborated on experiences of remote learning equivalent or superior to face-to-face experiences, associated with improved access to content and a reduction in operating costs. In a survey of six North American universities, promising distance learning practices, such as the

adaptation of the teaching portfolio, investments in infrastructure, and training. In the pandemic period and also in the post-COVID period19, EAD appears as a vital and efficient element in the learning process.

Materials and Methods

The research method applied to this article has an observational purpose, aiming the exploration of modern content based on the social and economic sciences. It is analyzed four important aspects related to the changes in human relationships and the international macroeconomic scenario after the COVID-19 pandemic.

Furthermore, in terms of the nature of the research, it is classified as applied and its approach is qualitative, as it allows an analysis of specific characteristics within a given circumstantial context [12]. Moreover, this research has an exploratory character, since it aims to clarify, analyze, and update concepts within a given context and explore an overview of current facts, through a wide investigation [12]. Concerning technical procedures, the methodology of this article uses bibliographic and documentary research [13]. In this context, scientific publications, articles, and documents from private organizations were used as a basis to allow the observational and logical analysis of this presented article.

Results and Discussion

We are experiencing an unprecedented global crisis due to the pandemic of COVID-19, causing dramatic changes to the world in early 2020, thereby changing the digital behavior of society around the globe. Right now, billions of people turn to connected devices to deal with life and interact in interpersonal relationships, in addition to working safely, where the focus remains on delivering results, but in an increasingly remote way. The internet then goes from a desirable item to an enabling item to a variety of services feasibility.

According to a recent survey conducted by Hootsuite [14] (Figure 3), there was a significant increase in digital and online activities in 2020. In this new context in Brazil, it will be extremely important to provide access to education, health, and communication for the entire community through the essential base element that is the Internet. Governments around the world have made interventions in the pandemic period to keep the crisis as stable and less impactful as possible in different sectors of the economy, from reducing taxes, granting emergency aid in the form of money, improving the health system, and even suspending temporarily loans and debts of citizens and companies.

In the same way that governments aim to promote the economy and the well-being of the country as a whole, it will be necessary to analyze the new basic element that is the internet and guarantee access for the whole society, especially in the most precarious regions, where access to the internet is still limiting, either due to the infrastructure of cities or the cost of providers.

In this perspective, the Brazilian government will be able to study the implementation of new economic and social projects aiming to assure this new basic element, as well as the current rural electrification program “Luz Para Todos”, which aims to allow access to electricity for more needy regions. The relevance of internet access in the Brazilian scenario is directly linked to several economic and social sectors, such as education, health, employability, or private companies.

The project feasibility must be associated with several actions linked to the expansion of the internet in the national territory, such as a free supply of the internet to society in most needy communities, direct intervention in the reduction of taxes of the current private internet providers allowing the acquisition of basic electronic and electrical components for the installation of new internet distribution centers, the launch of public notices aimed at the development of new national technologies, improvement in the infrastructures of cities that allow the provision of free

Figure 3. Internet connections chart.

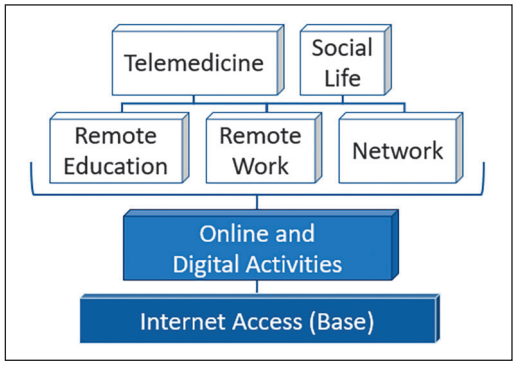
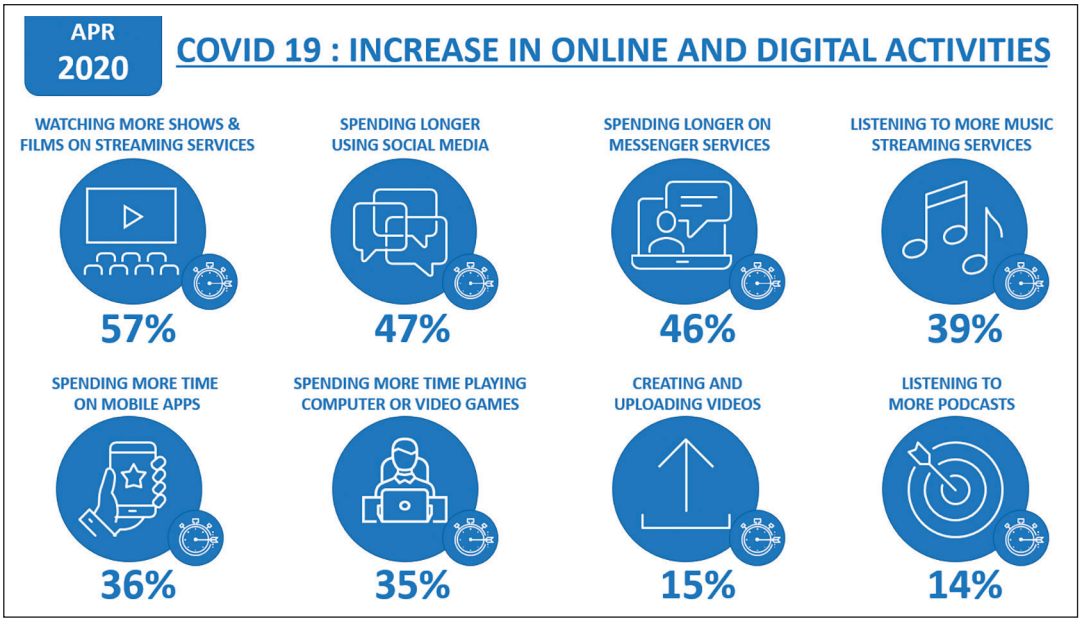


Figure 4. Activities impacted by COVID-19 pandemic in April.



internet in urban centers, also, to accelerate the implementation of the 5G network in the national territory.

Conclusion

In an increasingly connected world, where access to government resources for the most basic needs during a pandemic period relies on internet availability, it can be considered that those who are not connected to the network are socially invisible and are excluded from their rights. Despite the recent growth in the use of connected devices, this reality has not yet reached a large portion of the low-income Brazilian population. The recent economic crisis of 2014-2017 also played a major role in the negative impact of household access to the internet and delayed the digital revolution in Brazil, on average the demand for internet access decreased by 8 percentage points due to the situation of economic degradation. This decrease in demand has impacted families from different social classes who, in more adverse conditions, seek cheaper access solutions opting for the use of the internet only in places where this service is public and free. The digital revolution and investments in information and communication technology (ICT) demand a connected society, however, there is no connectivity without access to the internet and

there is no society without people. Investment in information and communication technologies (ICT) is directly associated with economic benefits, such as increased productivity, lower costs, new business opportunities, job creation, innovation, and increased trade (World Bank, 2018). The growth of ICTs also helps to create incentives to increase the participation of individuals and develop skills to deal with innovations in the digital world.

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The **VI International Symposium on Innovation and Technology (SIINTEC)** was focused on discussing challenges in science, technology and innovation after COVID-19. We had to make changes and find immediate solutions to keep people together, now this connection provides us the opportunity of having qualified participants from all over the world sharing and building knowledge.

The SIINTEC has been happening since 2015 and this year the VI edition, held by SENAI CIMATEC, occured from October 21 till 23. The main point of this event is providing the opportunity of reuniting the scientific and technological community to discuss innovation, researches and advances promoted by the pandemic period and draw applicable conclusions to society's new routine.

The December issue-2020 and February issue-2021 of **The Journal of Bioengineering and Technology Applied to Health (JBTH)** encompassed the approved articles of VI SIINTEC in the health area as presented below:

Title	Author(s)
Properties of Fibrous Composites with Polyester: A Comparative Analysis between Sisal Fiber and Pet	Matheus Vinicius Falcao Moreira
A Gamified Model for the Construction Site: A Solution to Motivate Construction Workers in Pandemic Time	Regina Maria Cunha Leite
Solar Resource Mapping in the Vale São Francisco da Bahia Using the Wrf-Solar Model	Carolina Sacramento Vieira
A Preliminary Evaluation of Vehicle Emissions from Pm10 in the Metropolitan Region of Salvador Using the Wrf-Smoke-Cmaq Models System	Katty Santos Da Silva
A Preliminary Study of the Evaluation of Air Quality for the Metropolitan Region of Salvador Using Photochemical Model Wrf-Chem	Anderson Da Silva Palmeira
Technological Prospective Study of Green Coffee Processing	Marcos Lage Cajazeira Ramos
Evaluation of the Deposition of Nanoparticles in the Human Respiratory Tract from the Burning of Diesel/Biodiesel/Additive	Clara Rodrigues Pereira
Senai Cimatec Contributions for the Strengthening of Brazilian	Carlos César Ribeiro Santos
Companies Using Lean Manufacturing in the Combat to Covid-19 Centesimal Composition and Physicochemical Properties of Oil Extracted From Moringa Oleifera Lam Seeds Grown in the State of Sergipe, Brazil	Milson Santos Barbosa
Identification and Study of a Promising Cyanbacteria Specie for Biotechnological Applications	Maria Teresa Araujo Pinheiro Menescal
Technology as Pillar for Essential Oil Green Extraction	Carlos Alberto Tosta Machado
Study on the Characteristics and Behavior of Expansive Soils	Paulo Cesar Burgos
Techniques and Methodologies Used for Analysis of Metals and Organic Compounds in Wastewaters, Graywater and Rainwater: A Brief Review	Vinícius Silva Dos Santos
Prediction of Density and Ultrasonic Velocity of Hydroxylic Compounds (C1-C6) as a Function of Temperature	Rebecca Da Silva Andrade
Characterization of *Arthrospira* Sp (Spirulina) Biomass when Grown in Alternative Hydroponic Waste Media: A Review	Yan Valdez Santos Rodrigues
Analysis of the Brazilian Manufacturers Presence in the Covid-19 Diagnostic Products Approved by Anvisa	Valdir Gomes Barbosa Júnior
Extraction and Characterization of Coffee Silverskin Oil with Potential Application for Enzymatic Synthesis of Fatty Acids	Danyelle Andrade Mota
Temperature Forecast for the Municipality of Mucuri	Flavio Santos Conterato
Importance of Occupational Health and Safety in Biotechnological Processes	Luma Mirely De Souza Brandão
Effect of Temperature on Physical Properties of Canadian Maple (*Acer Saccharum Marsh.*) Syrup	Marta Andrade Pires, Rebecca Da Silva Andrade, Miguel Iglesias
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Manuscript	Original	Review	Birief Comunication	Case Report	Editorial ; Letter to the Editor; Editor' s Corner	Innovative Medical Products	State-of-the-Art	Health Innovation Initiatives
Font Type	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial
Number of Words – Title	120	90	95	85	70	60	120	90
Font Size/Space-Title	12; double space	12; double space	12; double space	12; double space	12; double space	12; double space	12; double space	12; double space
Font Size/Space-Abstracts/Key Words and Abbreviations	10; single space	10; single space	10; single space	10; single space	-	-	10; single space	10; single space
Number of Words – Abstracts/Key Words	300/5	300/5	200/5	250/5	-	-	300/5	300/5
Font Size/Space-Text	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space
Number of Words – Text	5,000 including spaces	5,500 including spaces	2,500 including spaces	1,000 including spaces	1,000 including spaces	550 including spaces	5,000 including spaces	5,500 including spaces
Number of Figures	8 (title font size 12, double space)	3 (title font size 12, double space)	2 (title font size 12, double space)	2 (title font size 12, double space)	-	2 (title font size 12, double space)	8 (title font size 12, double space)	8 (title font size 12, double space)
Number of Tables/Graphic	7 title font size 12, double space	2 title font size 12, double space	2(title font size 12, double space)	1(title font size 12, double space)	-	-	7 title font size 12, double space	4 title font size 12, double space
Number of Authors and Co-authors*	15	10	5	10	3	3	15	10
References	20 (font size 10,single space	30(font size 10,single space	15 (font size 10,single space)	10 (font size 10,single space)	10 (font size 10,single space	5(font size 10,single space	20 (font size 10,single space	20

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