40% the number of elements, highlighting the formation of microspherocytes. This effect was time dependent and had a maximum at 20 minutes while at 40 minutes it returned to baseline. Heat-damaged RBCs did not show changes in osmotic fragility, hemolysis, and CBD-induced morphology. Together, these results suggest that CBD induces the formation of hemolytic vesicles via protein signaling from the cell membrane, with clinical relevance since the values of released hemoglobin were 10-15 times higher than the cut-off value (4mg%) for free hemoglobin in plasma.

INFECTOLOGÍA Y PARASITOLOGÍA

141. (002) **EARLY ANTIPARASITIC TREATMENT PREVENTS PROGRESSION OF CHAGAS DISEASE: RESULTS OF A LONG-TERM CARDIOLOGICAL FOLLOW-UP STUDY IN A PEDIATRIC POPULATION**

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Objective: To evaluate cardiac involvement in children after pharmacological treatment for Chagas disease (CD).
Methods: A descriptive study of a cohort of pediatric CD patients treated with benznidazole (Bz) or nifurtimox (Nf) was conducted by convenience sampling, 95 children with at least 6 years post-treatment follow-up and who attended a clinical visit between August 2015 and November 2019 were invited to participate in the study. They were evaluated with 24-hour Holter monitoring and speckle-tracking 2D echocardiogram (STE). As a control of the incidence of ECG non pathological findings a group of non-infected people were included.
Results: In enrolled treated patients: 24-hour Holter showed alterations in 3/95 (3%) patients, but only one was considered probably related to CD involvement. This patient presented a complete right bundle branch block (cRBBB). No contractility damage was found in 79/95 (83%) patients evaluated by STE.
In non-infected cardiological control group: 24-hour Holter showed alterations in 3/28 (10%) patients. No contractility damage was found in 25/28 patients evaluated by STE.
Benznidazole was prescribed in 87 patients and nifurtimox in 8 patients. Baseline parasitemia data was available for 65/95 patients. During follow-up, 59/61 (96%) treated patients achieved constant negative parasitemia evaluated by qPCR. A decrease in T.cruzi antibodies titers was observed and seroconversion occurred in 53/95 (56%) treated patients. These results showed a good efficacy of treatment in parasite clearance.
Conclusions: A good treatment response with a low incidence of cardiological lesions related to CD was observed. This suggests a protective effect of parasiticidal treatment on the development of cardiological lesions and highlights the importance of early treatment of infected children.

142. (004) **VALIDATION OF POOLED TESTING FOR SARS-CoV-2 USING DROPLET DIGITAL PCR**

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The outbreak of COVID-19 has become a public health emergency. Viral nucleic acid detection by reverse transcription PCR (RT-PCR) is the gold standard method for diagnosis of COVID-19. Droplet digital PCR (ddPCR) is a highly sensitive PCR technology based on the generation of 20,000 nanodrops per tube. This technology is rarely used in clinical laboratories, due to its higher cost when compared with PCR. Cost could be reduced by using pooled testing. A negative test result indicates that all individuals in the pool are negative while a positive result indicates that at least one individual is positive. Pooled testing may be particularly useful to communities with low prevalence of COVID-19. We proposed to validate the use of ddPCR to detect SARS-CoV-2 by pooling.
Throat swab samples of 1000 patients were collected and soaked in 2mL saline. RNA extraction was done using automatic magnetic extraction, columns extraction kits, and heat. Firstly, positive and negative samples were identified with RT-PCR, pools of different sizes were designed and ddPCR was performed. Data was analyzed with Quanta Soft analysis software v.1.7.4.0917 (Bio-Rad). This study was granted exception from bioethics committee approval as deidentified remnants samples were used.
We determined the specificity (we measured 100 negatives pools), the limit of detection (three independent octuplicates of the greatest dilution that it is positive) and the robustness of the method (the ability to withstand small but deliberate variations in method parameters by performing 20 repetitions changing the order of pooling and purification; and by measuring RNAs obtained using different extraction method). In the present work, we validated the use of pooled testing by combining up to 34 samples per pool.
We hope that such implementation of a pool test for SARS-CoV-19 would allow expanding current screening capacities, thereby enabling the expansion of detection in the community, as well as in close organic groups.

143. (012) **ERYTHROPOIETIN IN CHILDREN WITH HEMOLYTIC UREMIC SYNDROME: A RANDOMIZED CLINICAL TRIAL**

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Background: The efficacy of recombinant human erythropoietin (rHuEPO) in sparing red blood cell (RBC) transfusions in children with hemolytic uremic syndrome related to Shiga toxin-producing Escherichia coli (STEC-HUS) is uncertain.
Methods: We conducted a prospective, two-parallel-group randomized open controlled trial conducted at the Hospital General de Niños Pedro de Elizalde from December, 2018 to January, 2021 (ClinicalTrials.gov NCT03776851). We randomly assigned children with STEC-HUS to the rHuEPO group (subcutaneous rHuEPO 150 U/kg/week + RBC transfusion if hemoglobin ≤7 g/dLand/or hemodynamic instability) or to the usual- caregroup (RBC transfusion if hemoglobin ≤7 g/dL and/or hemodynamic instability). Primary outcome was the number of RBC transfusions received during the hospitalization. Secondary outcomes were to explore whether baseline EPO levels were deficient (according to the relation between observed and predicted level), to correlate selected acute phase parameters with the number of RBC transfusions, and to assess possible adverse events.
Results: Twelve patients per arm were included, all completed the trial. They were comparable at recruitment and coursed a similar acute disease. Median number of RBC transfusions was similar between groups (1.5, p=0.76). Most patients had appropriate baseline EPO levels, which did not correlate with the number of RBC transfusions (r 0.19, p=0.44). Conversely, baseline (r 0.73, p=0.032) and maximum lactic dehydrogenase levels (r 0.78, p=0.003), creatinine peak (r 0.71, p=0.03) and dialysis duration (r 0.7, p=0.04) correlated significantly with RBC requirements. No potential side effect was attributed to rHuEPO therapy.
Conclusion: Administration of rHuEPO did not reduce the number of RBC transfusions in children with STEC-HUS.

144. (019) **HYDATID FLUID FROM *ECHINOCOCCUS GRANULOSUS* INDUCES PHAGOPHORE AND AUTOPHAGOSOME FORMATION IN DENDRITIC CELLS THROUGH AN UPREGULATION OF BECLIN-1**

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Background: The cestode *Echinococcus granulosus* (Eg) is the etiological agent of cystic echinococcosis. This parasite develops cysts filled with hydatid fluid (HF) in the viscera of the intermediate host. Autophagy is a cellular catabolic process that plays a key role in the presentation of endogenous and exogenous proteins, promoting the activation of T cells. The aim of this work is to analyze if HF, constituted by a wide range of parasite proteins, could trigger autophagy in dendritic cells. **Methods:** HF was punctured from the hydatid cysts collected of infected cattle slaughtered. Murine BMDCs were cultured in RPMI 1640 medium, supplemented with FLT3-L. First, lysosome activity was evaluated using Acridine Orange, a fluorophore that can be trapped in acidic vesicular organelles. Then, autophagy induction was evaluated by FACS, qPCR, Confocal and Transmission Electron Microscopy. Rapamycin (20 nM) and chloroquine (100μM) were used to modulate autophagic flux. LC3-attachment to the autophagic membrane, were analyzed by stained DCs with anti LC3-β antibody (clone H50). **Results:** HF significantly increased acridine orange cytoplasmic accumulation compared to control cells (***p <0.001) and enhanced the effect of rapamycin (****p<0.0001). The ultrastructural analysis of TEM showed that in the presence of HF, DCs stimulate the formation of phagophores, double lipid membrane autophagosome, MVBs and autolysosomes. Also, HF-stimulated BMDCs significantly enhanced the mean fluorescence intensity of LC3-positive structures in comparison with unstimulated cells (*p<0.05, **p<0.01, ***p<0.001 HF-stimulated cells vs controls). Finally, we have observed that HF induces a significant increase in the transcriptional expression of LC3 and Beclin-1 (n=3, **p <0.01 vs control) and enhances the expression induced by rapamycin. **Conclusions:** These results suggest that HF of *Echinococcus granulosus* regulates gene expression to increase autophagy-related structures in DCs.

145. (129) **SERUM DETERMINATION OF TAU PROTEIN AS A POTENTIAL PREDICTIVE BIOMARKER OF ENCEPHALOPATHY ASSOCIATED WITH HEMOLYTIC UREMIC SYNDROME**

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Hemolytic uremic syndrome (HUS) is a foodborne disease caused by intoxication with Shiga toxin (Stx) produced by enterohemorrhagic E. coli (EHEC). HUS is characterized by microangiopathic hemolytic anemia, thrombocytopenia and renal failure. A variety of neurological alterations are often associated with poor prognosis and mortality risk, becoming the highest incidence of death due to HUS. In addition to Stx, EHEC is a gram-negative bacterium and thus releases LPS known to be involved in proinflammatory-related events which contributes significantly to the development of the disease. Early detection of neural serum biomarkers during the first days of bloody diarrhea manifestation, and prior HUS signs and symptoms, could be determinant to prevent the progress of the disease. We are currently studying the tau protein associated-neuronal microtubules. Its presence in blood as a neuronal damage consequence confirms a wide spectrum of brain insults. The aim of this work was to determine whether the neuronal tau protein can be considered an early serological biomarker of encephalopathy in the context of HUS. For this purpose, NIH-Swiss male mice were intravenously injected with vehicle, LPS (800ng), Stx2 (3.5ng, 1LD100) or a combination of Stx2 and LPS (Stx2+LPS, same previous amounts). After 1-and 2- days blood samples were collected to test by Elisa (Invitrogen, Viena, Austria) the detection of tau protein. One way ANOVA and Tukey post-hoc tests were employed for statistical analysis. A significant two-fold increase was determined after 2 days in the Stx-2+LPS group (p<0.05) with respect to the vehicle. Non-significant tau protein immunodetection was found in all groups after 1 day of treatment. Assuming that the murine death occurs after the fourth day of treatment, and that significant tau serum immunodetection was determined within 2 days, this protein could be used as a potential biomarker to prevent lethal encephalopathy associated to HUS.

146. (139) **BONE-MARROW DERIVED DENDRITIC CELLS FROM *TOXOPLASMA GONDII* CHRONICALLY INFECTED MICE EXHIBIT ALTERATIONS IN MONOCLONAL AND POLYCLONAL T CELL PRIMING**

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Rationale: We previously showed that splenocytes from chronic *T. gondii* infected mice have a diminished capacity to activate and differentiate OVA-specific Th1 and Th2 cells. Moreover, BMDCs from infected animals presented phenotypic alterations as shown by increases in CD80 and CD86 maturation markers and fewer secretion levels of IL-6 and IL-10, with no differences in IL-12. To extend these previous results, herein, we studied the ability of BMDC from chronically infected mice to activate and differentiate effector T cells. **Methods**: Bone-marrow derived dendritic cells (BMDCs) were obtained from naive and chronically infected mice, by culturing bone-marrow precursors for nine days with GM-CSF-conditioned medium. Afterward, BMDC were fed with OVA and matured during 18h with LPS. Subsequently, BMDC were cultured with DO11.10 OVA-specific CD4+T cells. Also, a mixed lymphocyte reaction (MLR) was performed by co-culturing BMDC with naive C57BL/6 mice splenocytes. **Results**: OVA specific CD4+ T cells co-cultured with BMDCs from infected mice showed lower levels of IFN-γ and IL-5 and increased levels of IL-10 (p<0.05). When analyzing polyclonal T cell responses in MLR assays, T lymphocytes incubated with BMDCs from infected mice showed decreased secretion of IFN-γ and IL-10 (p<0.01). No significant differences were observed for Th2 cytokines. **Conclusion**: The data obtained with BMDCs show that mice chronically infected with *T. gondii* present alterations of bone-marrow precursors. Interestingly, the diminished Th1/Th2 profiles observed in antigen specific co-culture assays are in line with results previously observed in splenocytes from *T. gondii* infected mice. These results suggest that infection with *T. gondii* results in long-lasting alterations in hematopoietic cells that could be involved in the lower susceptibility to developing allergic and autoimmune disorders.

147. (165) **THE NEW CAGE-LIKE PARTICLE ADJUVANT ISPA ENHANCE IMMUNITY OF AN EXPERIMENTAL VACCINE AGAINST CHRONIC *TOXOPLASMA GONDII* INFECTION IN MICE**

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Toxoplasmosis is a disease that affects 30% of the world's population. At present, there are no pharmacological treatments that eliminate the parasite or vaccines that confer protection to the host. The aim of the present work was to study the immunogenicity of vaccine formulations containing a new cage-like particle adjuvant (ISPA) in combination with *T. gondii* recombinant proteins.

METHODS: C57BL/6 mice were intradermally immunized 3-times with a 2-week interval with rGRA7, rTgPF, rTgPI-1 or rROP2 in com-