

Cuban Research in Current International Journals

A multiplex PCR for the detection of *Fasciola hepatica* in the intermediate snail host *Galba cubensis*.

Alba A, Vázquez AA, Hernández H, Sánchez J, Marcet R, Figueredo M, et al. Vet Parasitol. 2015 May 15. pii: S0304-4017(15)00248-4.

DOI: 10.1016/j.vetpar.2015.05.012. [Epub ahead of print]

Fasciolosis is a snail-borne trematode infection that has re-emerged as a human disease, and is considered a significant problem for veterinary medicine worldwide. The evaluation of the transmission risk of fasciolosis as well as the efficacy of the strategies for its control could be carried out through epidemiological surveillance of the snails that act as intermediate hosts of the parasites. The present study aimed to develop the first multiplex PCR to detect *Fasciola hepatica* in *Galba cubensis*, an important intermediate host of the parasite in the Americas and especially in the Caribbean basin. The multiplex PCR was optimized for the amplification of a 340bp fragment of the second internal transcribed spacer (ITS-2) of *F. hepatica* rDNA, while another set of primers was designed and used to amplify a conserved segment of the nuclear 18S rDNA of the snail (451bp), as an internal control of the reaction. The assay was able to detect up to 100pg of the parasite even at high concentrations of snail DNA, an analytical sensitivity that allows the detection of less than a single miracidium, which is the minimal biological infestation unit. A controlled laboratory-reared *G. cubensis* - *F. hepatica* system was used for the evaluation of the developed multiplex PCR, and 100% sensitivity and specificity was achieved. This assay constitutes a novel, useful and suitable technique for the survey of fasciolosis transmission through one of the main intermediate hosts in the Western hemisphere.

Anatomic and functional connectivity relationship in autistic children during three different experimental conditions.

Machado C, Estévez M, Rodríguez R, Leisman G, Melillo R, Chinchilla M, et al. Brain Connectivity. 2015 Jun 7. [Epub ahead of print]

A group of 21 autistic children was studied for determining the relationship between the anatomic (AC) vs. functional (FC) connectivity, considering short-range and long-range brain networks. AC was assessed by the DW-MRI technique and FC by EEG coherence calculation, in three experimental conditions: basal, watching a popular cartoon with audio (V-A), and with muted audio track (VwA). For short-range connections, basal records, statistical significant correlations were found for all EEG bands in the left hemisphere, but no significant correlations were found for fast EEG frequencies in the right hemisphere. For the V-A condition, significant correlations mainly diminished for the left hemisphere; for the right hemisphere, no significant correlations were found for the fast EEG frequency bands. For the VwA condition, significant correlations for the rapid EEG frequencies mainly disappeared for the right hemisphere. For long-range connections, basal records showed similar correlations both hemispheres. For the right hemisphere, significant correlations incremented to all EEG bands for the V-A condition, but these significant correlations disappeared for the fast EEG frequencies in the VwA condition. It appears that in a resting-state condition, AC is better associated with functional connectivity for short-range connections in the left hemisphere. V-A experimental condition enriches AC and FC association for long-range connections in the right hemisphere. This might be related to an effective connectivity improvement due to full video stimulation (visual and auditory). An impaired audio-visual interaction in the right hemisphere might explain why significant correlations disappeared for the fast EEG frequencies in the VwA experimental condition.

Assessment of the impact of manufacturing changes on the physicochemical properties and biological activity of Her1-ECD vaccine during product development.

García Duardo K, Prieto Curbelo Y, Raymond Pous J, Rabasa Legón EY, Ramírez BS, Hernández KR, et al. Vaccine. 2015 May 21. pii: S0264-410X(15)00639-8.

DOI: 10.1016/j.vaccine.2015.05.018. [Epub ahead of print]

Vaccine preparations based on the extracellular domain of Her1 (Her1-ECD) have demonstrated, in vitro and in vivo, a potent antimetastatic effect on EGFR+ Lewis lung carcinoma model, while associated side effects were absent. The Her1-ECD is a glycoprotein with a molecular weight of 105kDa and has 11 potential sites for N-glycosylation. Currently Her1-ECD based vaccine has been evaluated in patients with hormone refractory prostate cancer. Her1-ECD molecule used for in clinical trials was obtained from culture supernatant of HEK 293 transfectomes used the protein free culture media and is purified by immunoaffinity chromatography. In order to increase the cell growth and productivity, new defined culture media have been developed (alternative culture media) in Her1-ECD vaccine production process. In this work, a comparability study was performed to evaluate the impact of process changes in the characteristics physico-chemical and biologicals of the Her1-ECD protein and the degree of similitude between both variants. Techniques such as: SDS-PAGE, SEC-HPLC,

isoelectric point, peptide mapping, mass spectrometric, SCX-HPLC, oligosaccharide map, ELISA and flow cytometric were used with this aim. Results indicated that this process change decreases the degree of sialylation of the protein but does not affect its biological activity (measured as titers of Abs and recognition for A431 cell line).

Bond-based bilinear indices for computational discovery of novel trypanosomical drug-like compounds through virtual screening.

Castillo JA, del Toro O, Vega MC, Rolón M, Rojas A, Casañola GM, et al. Eur J Med Chem. 2015 May 26;96:238–44.

Two-dimensional bond-based bilinear indices and linear discriminant analysis are used in this report to perform a quantitative structure-activity relationship study to identify new trypanosomicidal compounds. A data set of 440 organic chemicals, 143 with antitrypanosomal activity and 297 having other clinical uses, is used to develop the theoretical models. Two discriminant models, computed using bond-based bilinear indices, are developed and both show accuracies higher than 86% for training and test sets. The stochastic model correctly identifies nine out of eleven compounds of a set of organic chemicals obtained from our synthetic collaborators. The in vitro antitrypanosomal activity of this set against epimastigote forms of *Trypanosoma cruzi* is assayed. Both models show a good agreement between theoretical predictions and experimental results. Three compounds showed IC₅₀ values for epimastigote elimination (AE) lower than 50 μ M, while for the benznidazole the IC₅₀ = 54.7 μ M which was used as reference compound. The value of IC₅₀ for cytotoxicity of these compounds is at least 5 times greater than their value of IC₅₀ for AE. Finally, we can say that, the present algorithm constitutes a step forward in the search for efficient ways of discovering new antitrypanosomal compounds.

Changes in arterial stiffness, carotid intima-media thickness, and epicardial fat after L-thyroxine replacement therapy in hypothyroidism.

Del Busto-Mesa A, Cabrera-Rego JO, Carrero-Fernández L, Hernández-Roca CV, González-Valdés JL, de la Rosa Pazos JE. Endocrinol Nutr. 2015 Apr 13. DOI: 10.1016/j.endonu. [Epub ahead of print]

Objective To assess the relationship between primary hypothyroidism and subclinical atherosclerosis and its potential changes with L-thyroxine replacement therapy. **Methods** A prospective cohort study including 101 patients with primary hypothyroidism and 101 euthyroid patients as controls was conducted from July 2011 to December 2013. Clinical, anthropometrical, biochemical, and ultrasonographic parameters were assessed at baseline and after one year of L-thyroxine replacement therapy. **Results** At baseline, hypothyroid patients had significantly greater values of blood pressure, total cholesterol, VLDL cholesterol, left ventricular mass, epicardial fat, and carotid intima-media thickness as compared to controls. Total cholesterol, VLDL cholesterol, ventricular diastolic function, epicardial fat, carotid intima-media thickness, carotid local pulse wave velocity, pressure strain elastic modulus, and β arterial stiffness index showed a significant and positive correlation with TSH levels. After one year of replacement therapy, patients with hypothyroidism showed changes in total cholesterol, VLDL cholesterol, TSH, carotid intima-media thickness, and arterial stiffness parameters. **Conclusions** Primary hypothyroidism is characterized by an increased cardiovascular risk. In these patients, L-thyroxine replacement therapy for one year is related to decreased dyslipidemia and improvement in markers of subclinical carotid atherosclerosis.

Common variants at the 9q22.33, 14q13.3 and ATM loci, and risk of differentiated thyroid cancer in the Cuban population.

Pereda CM, Lesueur F, Pertesi M, Robinot M, Lence-Anta JJ, Turcios S, et al. BMC Genet. 2015 Mar;16:22. DOI: 10.1186/s12863-015-0180-5.

Background The incidence of differentiated thyroid carcinoma (DTC) in Cuba is low and the contribution of host genetic factors to DTC in this population has not been investigated so far. Our goal was to assess the role of known risk polymorphisms in DTC cases living in Havana. We genotyped five polymorphisms located at the DTC susceptibility loci on chromosome 14q13.3 near NK2 homeobox 1 (NKX2-1), on chromosome 9q22.33 near Forkhead factor E1 (FOXE1) and within the DNA repair gene Ataxia-Telangiectasia Mutated (ATM) in 203 cases and 212 age- and sex- matched controls. Potential interactions between these polymorphisms and other DTC risk factors such as body surface area, body mass index, size, ethnicity, and, for women, the parity were also examined. **Results** Significant association with DTC risk was found for rs944289 near NKX2-1 (OR per A allele = 1.6, 95% CI: 1.2-2.1), and three polymorphisms near or within FOXE1, namely rs965513 (OR per A allele = 1.7, 95% CI: 1.2-2.3), rs1867277 in the promoter region of the gene (OR per A allele = 1.5, 95% CI: 1.1-1.9) and the poly-alanine tract expansion polymorphism rs71369530 (OR per Long Allele = 1.8, 95% CI: 1.3-2.5), only the 2 latter remaining significant when correcting for multiple tests. Overall, no association between DTC and the coding SNP D1853N (rs1801516) in ATM (OR per A Allele = 1.1, 95% CI: 0.7-1.7) was

seen. Nevertheless women who had 2 or more pregnancies had a 3.5-fold increase in risk of DTC if they carried the A allele (OR 3.5, 95% CI: 3.2-9.8) as compared to 0.8 (OR 0.8, 95% CI: 0.4-1.6) in those who had fewer than 2. **Conclusions** We confirmed in the Cuban population the role of the loci previously associated with DTC susceptibility in European and Japanese populations through genome-wide association studies. Our results on ATM and the number of pregnancies raise interesting questions on the mechanisms by which oestrogens, or other hormones alter the DNA damage response and DNA repair through the regulation of key effector proteins such as ATM. Due to the small size of our study and to multiple tests; all these results warrant further investigation.

Comparative proteomics analysis of the antitumor effect of CIGB-552 peptide in HT-29 colon adenocarcinoma cells.

Núñez de Villavicencio-Díaz T, Ramos Gómez Y, Oliva Argüelles B, Fernández Masso JR, Rodríguez-Ulloa A, Cruz García Y, et al. J Proteomics. 2015 May 24;126:163–71.

The second generation peptide CIGB-552 has a pro-apoptotic effect on H460 non-small cell lung cancer cells and displays a potent cytotoxic effect in HT-29 colon adenocarcinoma cells though its action mechanism is ill defined. Here, we present the first proteomic study of peptide effect in HT-29 cells using subcellular fractionation, protein and peptide fractionation by DF-PAGE and LC-MS/MS peptide identification. In particular, we explored the nuclear proteome of HT-29 cells at a 5h treatment identifying a total of 68 differentially modulated proteins, 49 of which localize to the nucleus. The differentially modulated proteins were analyzed following a system biology approach. Results pointed to a modulation of apoptosis, oxidative damage removal, NF- κ B activation, inflammatory signaling and of cell adhesion and motility. Further Western blot and flow-cytometry experiments confirmed both pro-apoptotic and anti-inflammatory effects of CIGB-552 peptide in HT-29 cells.

Dietary flaxseed independently lowers circulating cholesterol and lowers it beyond the effects of cholesterol-lowering medications alone in patients with peripheral artery disease.

Edel AL, Rodríguez-Leyva D, Maddaford TG, Caligiuri SP, Austria JA, Weighell W, et al. J Nutr. 2015 Apr;145(4):749–57.

Background Dietary flaxseed lowers cholesterol in healthy subjects with mild biomarkers of cardiovascular disease (CVD). **Objective** The aim was to investigate the effects of dietary flaxseed on plasma cholesterol in a patient population with clinically significant CVD and in those administered cholesterol-lowering medications (CLMs), primarily statins. **Methods** This double-blind, randomized, placebo-controlled trial examined the effects of a diet supplemented for 12 mo with foods that contained either 30 g of milled flaxseed [milled flaxseed treatment (FX) group; $n = 58$] or 30 g of whole wheat [placebo (PL) group; $n = 52$] in a patient population with peripheral artery disease (PAD). Plasma lipids were measured at 0, 1, 6, and 12 mo. **Results** Dietary flaxseed in PAD patients resulted in a 15% reduction in circulating LDL cholesterol as early as 1 mo into the trial ($P = 0.05$). The concentration in the FX group (2.1 ± 0.10 mmol/L) tended to be less than in the PL group (2.5 ± 0.2 mmol/L) at 6 mo ($P = 0.12$), but not at 12 mo ($P = 0.33$). Total cholesterol also tended to be lower in the FX group than in the PL group at 1 mo (11%, $P = 0.05$) and 6 mo (11%, $P = 0.07$), but not at 12 mo ($P = 0.24$). In a subgroup of patients taking flaxseed and CLM ($n = 36$), LDL-cholesterol concentrations were lowered by $8.5\% \pm 3.0\%$ compared with baseline after 12 mo. This differed from the PL + CLM subgroup ($n = 26$), which increased by $3.0\% \pm 4.4\%$ ($P = 0.030$) to a final concentration of 2.2 ± 0.1 mmol/L. **Conclusions** Milled flaxseed lowers total and LDL cholesterol in patients with PAD and has additional LDL-cholesterol-lowering capabilities when used in conjunction with CLMs. This trial was registered at clinicaltrials.gov as [NCT00781950](https://clinicaltrials.gov/ct2/show/study?term=NCT00781950).

Experimental studies of a vaccine formulation of recombinant human VEGF antigen with aluminum phosphate.

Pérez Sánchez L, Morera Díaz Y, Beguet-Romero M, Ramsés Hernández G, Rodríguez Y, Castro Velazco J, et al. Hum Vaccine Immunother. 2015 Apr;18. [Epub ahead of print]

CIGB-247 is a cancer vaccine that is a formulation of a recombinant protein antigen representative of the human vascular endothelial growth factor (VEGF) with a bacterially-derived adjuvant (VSSP). The vaccine has shown an excellent safety profile in mice, rats, rabbits, non-human primates and in recent clinical trials in cancer patients. Response to the vaccine is characterized by specific antibody titers that neutralize VEGF/VEGFR2 binding and a cytotoxic tumor-specific response. To expand our present anti-VEGF active immunotherapy strategies, we have now studied in mice and non-human primates the effects of vaccination with a formulation of our recombinant VEGF antigen and aluminum phosphate adjuvant (hereafter denominated CIGB-247-A). Administered bi-weekly, CIGB-247-A produces high titers of anti-VEGF IgG blocking antibodies in two mice strains. Particularly in BALB/c, the treatment impaired subcutaneous F3II

mammary tumor growth and reduced the number of spontaneous lung macro metastases, increasing animals' survival. Spleen cells from specifically immunized mice directly killed F3II tumor cells in vitro. CIGB-247-A also showed to be immunogenic in non-human primates, which developed anti-VEGF blocking antibodies and the ability for specific direct cell cytotoxic responses, all without impairing the healing of deep skin wounds or other side effect. Our results support consideration of aluminum phosphate as a suitable adjuvant for the development of new vaccine formulations using VEGF as antigen.

Exploring the "weight" of food cravings and thought suppression among Cuban adults.

Rodríguez-Martín BC, Gil-Pérez P, Pérez-Morales I. *Eat Weight Disord.* 2015 Jun;20(2):249–56.

Purpose The current study aimed to analyze individual differences on food cravings, intrusive-related thoughts and its suppression between normal weight and overweight/obese Cuban adults. **Methods** Participants were 1,184 individuals from general population, aged between 18 and 64 years ($M = 32.89$; $SD = 12.87$), with 69.1 % females. All participants answered a set of questionnaires and provided demographic, anthropometric and clinical data. **Results** Overweight/obese individuals had higher mean scores than normal weight individuals on food cravings (including its nine dimensions) and food and body weight/shape thought suppression. Large effect sizes were found for body weight/shape thoughts suppression and lack of control over eating, where overweight and obese individuals showed the highest scores. This trend was also found for food thoughts suppression, food cravings trait, cue-dependent eating, preoccupation with food and guilty feelings, with effect sizes from medium to large. Finally, medium effect sizes were observed for intention to eat and negative affect. **Conclusion** Overweight/obese individuals experienced more food cravings and food and body weight/shape thought suppression than normal weight individuals among Cuban adults.

Genetic Diversity of the Hepatitis B Virus Strains in Cuba: Absence of West-African Genotypes despite the Transatlantic Slave Trade.

Rodríguez Lay LA, Corredor MB, Villalba MC, Frómeta SS, Wong MS, Valdés L, et al. *PLoSOne.* 2015 May 15;10(5):e0125052. DOI: 10.1371/journal.pone.0125052.

Cuba is an HBsAg low-prevalence country with a high coverage of anti-hepatitis B vaccine. Its population is essentially the result of the population mix of Spanish descendants and former African slaves. Information about genetic characteristics of hepatitis B virus (HBV) strains circulating in the country is scarce. The HBV genotypes/subgenotypes, serotypes, mixed infections, and S gene mutations of 172 Cuban HBsAg and HBV-DNA positive patients were determined by direct sequencing and phylogenetic analysis. Phylogenetic analysis of HBV S gene sequences showed a predominance of genotype A (92.4%), subgenotype A2 (84.9%) and A1 (7.6%). Genotype D (7.0%) and subgenotype C1 (0.6%) were also detected but typical (sub) genotypes of contemporary West-Africa (E, A3) were conspicuously absent. All genotype A, D, and C strains exhibited sequence characteristics of the adw2, ayw2, and adr_q serotypes, respectively. Thirty-three (19.1%) patients showed single, double, or multiple point mutations inside the Major Hydrophilic domain associated with vaccine escape; eighteen (10.5%) patients had mutations in the T-cell epitope (amino acids 28-51), and there were another 111 point mutations downstream of the S gene. One patient had an HBV A1/A2 mixed infection. This first genetic study of Cuban HBV viruses revealed only strains that were interspersed with strains from particularly Europe, America, and Asia. The absence of genotype E supports previous hypotheses about an only recent introduction of this genotype into the general population in Africa. The presence of well-known vaccine escape (3.5%) and viral resistance mutants (2.9%) warrants strain surveillance to guide vaccination and treatment strategies.

Height, zinc and soil-transmitted helminth infections in schoolchildren: a study in Cuba and Cambodia.

De Gier B, Mpoabanzi L, Vereecken K, van der Werff SD, D'Haese PC, Fiorentino M, et al. *Nutrients.* 2015 Apr 20;7(4):3000–10.

Soil-transmitted helminth (STH) infections and zinc deficiency are often found in low- and middle-income countries and are both known to affect child growth. However, studies combining data on zinc and STH are lacking. In two studies in schoolchildren in Cuba and Cambodia, we collected data on height, STH infection and zinc concentration in either plasma (Cambodia) or hair (Cuba). We analyzed whether STH and/or zinc were associated with height for age z-scores and whether STH and zinc were associated. In Cuba, STH prevalence was 8.4%; these were mainly *Ascaris lumbricoides* and *Trichuris trichiura* infections. In Cambodia, STH prevalence was 16.8%, mostly caused by hookworm. In Cuban children, STH infection had a strong association with height for age ($aB-0.438$, $p = 0.001$), while hair zinc was significantly associated with height for age only in STH uninfected children. In Cambodian children, plasma zinc was associated with height for age ($aB-0.033$, $p = 0.029$), but STH infection was not. Only in Cambodia, STH infection showed an association

with zinc concentration (aB-0.233, $p = 0.051$). Factors influencing child growth differ between populations and may depend on prevalences of STH species and zinc deficiency. Further research is needed to elucidate these relationships and their underlying mechanisms.

Law and the medicalization of maternity, paternity and childbirth in Cuba.

García-Jordá D, Díaz-Bernal Z, Acosta-Álamo M. Rev Cienc Salud. 2015 Jun;10(2):207–21.

The childbirth is a transcendental moment in personal and family life. During this event, some aspects are revised related with femininity and masculinity. **Objective** From this perspective, was carried out this research with the purpose of understand how the maternity representation, the medical system that attend childbirth and the laws influence in the conception and practice of maternity and paternity during the pregnant, labor, and postpartum. **Methodology** Some qualitative techniques were used, as interviews and open observation to women and their partners (spouses or common laws) who were attended in three hospitals of Havana. **Results** The results show as the traditional gender roles are reinforced by the laws, institutional regulations and the medical practice in hospitals. This traditional view has been that women take on most child care responsibilities and men are relegated of this. Parallel with this, a new form of masculinity emerges as a result of the increasing of participation of men in the event and in household activities and the externalization of feelings when they know their children. **Conclusion** New gender policies are needed in order to deconstruct the patriarchal culture and develop egalitarian and democratic relationships in Cuban society.

Low detection of *Vibrio cholerae* carriage in healthcare workers returning to 12 Latin American countries from Haiti.

Llanes R, Somarriba L, Hernández G, Bardaji Y, Aguila A, Mazumder RN. Epidemiol Infect. 2015 Apr;143(5):1016–9.

This investigation was undertaken to characterize the prevalence of intestinal *Vibrio cholerae* in healthcare workers (HCWs) returning from Haiti due to the ongoing cholera epidemic. Eight hundred and fifty asymptomatic HCWs of the Cuban Medical Brigade, who planned to leave Haiti, were studied by laboratory screening of stool culture for *V. cholerae*. A very low percentage (0.23%) of toxigenic *V. cholerae* serogroup O1, serotype Ogawa was found. To the best of our knowledge, this study represents the largest reported screening study for *V. cholerae* infection in asymptomatic HCWs returning from a cholera-affected country. Cholera transmission to health personnel highlights a possible risk of transmitting cholera during mobilization of the population for emergency response. Aid workers are encouraged to take precautions to reduce their risk for acquiring cholera and special care should be taken by consuming safe water and food and practising regular hand washing.

Maternal oxidant and antioxidant status in the third trimester of gestation and its relation to the birth-weight.

Cruz Manzano E, Elías Postigo O, Corría Osorio J, León Moreno M, Milanés Ojea M, Valdés Izaguirre LE. Obstetrics Gynecol. 2015. 10 p.
DOI: 10.5171/2015.858253.

The aim of this study was to determine if the behavior of redox status indicators and their relation to birth weight, previously reported, was dependent only on the period between 30 and 36 weeks gestation, or if it was a characteristic of the third trimester. The present study was done in pregnant women between 25 and 29 and between 30 and 36 weeks of gestation; in addition, a reference group with non-pregnant women was formed. Ferric reducing potential, uric acid, albumin, vitamins C, E, A, erythrocyte reduced glutathione concentrations, total extracellular superoxide dismutase and catalase activity were measured as antioxidant indicators, and serum malondialdehyde plus 4-hydroxynonenal concentration as a lipoperoxidation indicator. No significant differences between the values of the indicators of redox status between the two groups of pregnant women were found. Significantly lower maternal serum Ferric Reducing Potential and albumin concentration were noted in pregnant women between 25 to 29 and 30 to 36 weeks, respectively. However, extracellular superoxide dismutase activity and erythrocyte reduced glutathione concentration were increased in gestational groups compared to non-pregnant group. Malondialdehyde concentrations plus 4-hydroxy-nonenal correlated significantly and inversely with birth-weight, while erythrocyte reduced glutathione correlated significantly and directly in the two groups of pregnant women; however, the activity of the total extracellular superoxide dismutase correlated with birth-weight only in the group of pregnant women between 30 and 36 weeks. We conclude that the behavior of the indicators of maternal redox status and its influence on birth-weight is similar for both groups of pregnant women with gestational age studied.

New genetic variants of influenza A(H1N1)pdm09 detected in Cuba during 2011-2013.

Arencibia A, Acosta B, Muné M, Valdés O, Fernández L, Medina I, et al. Infect Genet Evol. 2015 Jun;32:322–6. DOI: 10.1016/j.meegid.2015.03.028.

Influenza A(H1N1)pdm09 virus has evolved continually since its emergence in 2009. For influenza virus strains, genetic changes occurring in HA1 domain of the hemagglutinin cause the emergence of new variants. The aim of our study is to establish genetic associations between 35 A(H1N1)pdm09 viruses circulating in Cuba in 2011-2012 and 2012-2013 seasons, and A/California/07/2009 strain recommended by WHO as the H1N1 component of the influenza vaccine. The phylogenetic analysis revealed the circulation of clades 3, 6A, 6B, 6C and 7. Mutations were detected in the antigenic site or in the receptor-binding domains of HA1 segment, including S174P, S179N, K180Q, S202T, S220T and R222K. Substitutions S174P, S179N, K180Q and R222K were detected in Cuban strains for the first time.

Optimizing CIGB-300 intralesional delivery in locally advanced cervical cancer.

Sarduy MR, García I, Perera A, Torres LA, Valenzuela CM, Baladrón I, et al. Br J Cancer. 2015 May 12;112(10):1636–43.

Background We conducted a phase 1 trial in patients with locally advanced cervical cancer by injecting 0.5 ml of the CK2-antagonist CIGB-300 in two different sites on tumours to assess tumour uptake, safety, pharmacodynamic activity and identify the recommended dose. **Methods** Fourteen patients were treated with intralesional injections containing 35 or 70 mg of CIGB-300 in three alternate cycles of three consecutive days each before standard chemoradiotherapy. Tumour uptake was determined using (99)Tc-radiolabelled peptide. In situ B23/nucleophosmin was determined by immunohistochemistry. **Results** Maximum tumour uptake for CIGB-300 70-mg dose was significantly higher than the one observed for 35 mg: 16.1±8.9 vs 31.3±12.9 mg (P=0.01). Both, AUC24h and biological half-life were also significantly higher using 70 mg of CIGB-300 (P<0.001). Unincorporated CIGB-300 diffused rapidly to blood and was mainly distributed towards kidneys, and marginally in liver, lungs, heart and spleen. There was no DLT and moderate allergic-like reactions were the most common systemic side effect with strong correlation between unincorporated CIGB-300 and histamine levels in blood. CIGB-300, 70 mg, downregulated B23/nucleophosmin (P=0.03) in tumour specimens. **Conclusion** Intralesional injections of 70 mg CIGB-300 in two sites (0.5 ml per injection) and this treatment plan are recommended to be evaluated in phase 2 studies.

Objective measurements of image quality in synchrotron radiation phase-contrast imaging versus digital mammography.

Ruiz-González Y, Pérez-Díaz M, Martínez-Aguila D, Díaz Barreto M, Fleitas I, Mora-Machado R, et al. Int J Comput Assist Radiol Surg. 2015 Jun 20. [Epub ahead of print]

Purpose Phase-contrast mammography with synchrotron radiation is an innovative X-ray imaging practice that improves the identification of breast lesions. Previous studies have proven the superiority of the mammography images taken in the phase-contrast modality using synchrotron radiation beams as compared with images taken in conventional mammography by subjective analyses. However, to our knowledge, no previous study has compared different acquisition systems in order to quantify this improvement by means of objective robust indicators. In this research, we intend to quantify the superiority of phase-contrast imaging by means of objective metrics of image quality. **Methods** Images from the American College of Radiology Mammographic Accreditation Phantom were obtained at hospitals, in two digital mammography equipment and at the Elettra synchrotron radiation facility (Trieste, Italy), using free space propagation phase-contrast modality. Regions of interest were selected to analyze image quality at the fibers (phase object) and masses (area object) simulated on the phantom by means of the signal-to-noise ratio, the figure of merit, the contrast and the edge visibility. **Results** The image contrast and edge visibility were significantly higher at the phase-contrast modality as compared with digital mammography equipment. The figure of merit using phase-contrast modality was higher for the fibers and comparable for the masses. **Conclusion** The results showed an improvement of the contrast and edge visibility in phase-contrast images. These improvements may be important in the detection of small lesions and details.

Panayiotopoulos syndrome and diffuse paroxysms as the first EEG manifestation at clinical onset: a study of nine patients.

Caraballo RH, Pasteris MC, Portuondo E, Fortini PS. Epileptic Disord. 2015 Jun;17(2):143–9. [Epub ahead of print]

To present a retrospective study of nine children with Panayiotopoulos syndrome associated with diffuse spikes and waves as the sole EEG manifestation at onset. Charts of children with typical clinical criteria of

Panayiotopoulos syndrome, electroclinically followed between February 2000 and February 2012, were reviewed. Among 150 patients who met the electroclinical criteria of Panayiotopoulos syndrome, we identified nine children who presented with the typical clinical manifestations but who, on EEG, only had diffuse paroxysms at onset that continued along the course of the syndrome. In three, in addition to the diffuse paroxysms, focal spikes appeared later. From a clinical point of view, other features were otherwise unremarkable. Diffuse spike-and-wave discharges were observed in all patients when awake and during sleep (100%). Later, three children also had focal spikes during sleep, which were occipital in one, frontal in one, and temporo-occipital in the remaining patient. Spikes were activated by sleep in all three cases. During disease evolution, no particular electroclinical pattern was observed. Two patients who received clobazam and carbamazepine, respectively, did not respond well to the drugs and valproic acid was added with excellent seizure control. Outcome was good. We present evidence that patients with Panayiotopoulos syndrome may have diffuse discharges at onset as the sole EEG manifestation, which last throughout the course of the syndrome. In some, focal paroxysms developed later. The course was benign. In our group of patients, clinical features and evolution were similar to those of typical cases of Panayiotopoulos syndrome.

Recombinant streptokinase vs hydrocortisone suppositories in acute hemorrhoids. A randomized controlled trial.

Hernández-Bernal F, Castellanos-Sierra G, Valenzuela-Silva CM, Catasús-Alvarez KM, Martínez-Serrano O, Lazo-Diogo OC, et al. World J Gastroenterol. 2015 Jun 21;21(23):7305–12.

Aim To compare the efficacy and safety of recombinant streptokinase (rSK) vs hydrocortisone acetate-based suppositories in acute hemorrhoidal disease. **Methods** A multicenter (11 sites), randomized (1:1:1), open, controlled trial with parallel groups was performed. All participating patients gave their written, informed consent. After inclusion, patients with acute symptoms of hemorrhoids were centrally randomized to receive, as outpatients, by the rectal route, suppositories of rSK 200000 IU of one unit every 8 h (first 3 units) and afterwards every 12 h until 8 administrations were completed (schedule A), one unit every 8 h until 6 units were completed (schedule B), or 25 mg hydrocortisone acetate once every 8 h up to a maximum of 24 administrations. Evaluations were performed at 3, 5, and 10 d post-inclusion. The main end-point was the 5th day response (disappearance of pain and bleeding, and $\geq 70\%$ reduction of the lesion size). Time to response and need for thrombectomy were secondary efficacy variables. Adverse events were also evaluated. **Results** Groups were homogeneous with regards to demographic and baseline characteristics. Fifth day complete response rates were 156/170 (91.8%; 95%CI: 87.3-96.2), 155/170 (91.2%; 95%CI: 86.6%-95.7%), and 46/170 (27.1%; 95%CI: 20.1%-34.0%) with rSK (schedule A and B) and hydrocortisone acetate suppositories, respectively. These 64.6% and 63.9% differences (95%CI: 56.7%-72.2% and 55.7%-72.0%) were highly significant ($P < 0.001$). This advantage was detected since the early 3rd day evaluation (68.8% and 64.1% vs 7.1% for the rSK and active control groups, respectively; $P < 0.001$) and was maintained even at the late 10th day assessment (97.1% and 93.5% vs 67.1% for rSK and hydrocortisone acetate, respectively; $P < 0.001$). Time to response was 3 d (95%CI: 2.9-3.1) for both rSK groups and 10 d (95%CI: 9.3-10.7) in the hydrocortisone acetate group. This difference was highly significant ($P < 0.001$). All subgroup stratified analyses (with or without thrombosis and hemorrhoid classification) showed a statistically significant advantage for the rSK groups. Thrombectomy was necessary in 4/251 and 14/133 patients with baseline thrombosis in the rSK and hydrocortisone acetate groups, respectively ($P < 0.001$). There were no adverse events attributable to the experimental treatment. **Conclusion** rSK suppositories showed a significant advantage over a widely-used over-the-counter hydrocortisone acetate preparation for the treatment of acute hemorrhoidal illness, as well as having an adequate safety profile.

Rectal insufflation of ozone attenuates chronic oxidative stress in elderly patients with cardiovascular diseases.

Wilkins-Pérez I, Delgado-Roche L, Barrios JM, Fábregas-Popoco GB. Oxid Antioxid Med Sci. 2015;4(1):23–7.

Objective Aging is a biological inevitable process, characterized by a progressive deterioration of physiological functions and metabolism, ultimately leading to death, meanwhile cardiovascular diseases are the most common cause of death in aged subjects. Chronic oxidative stress contributes to age-associated cardiovascular dysfunctions and diseases. Because ozone therapy can modulate the antioxidant system the aim of this study was to investigate the therapeutic efficacy of ozone in aged patients with cardiovascular diseases. **Methods** A randomized controlled clinical trial was performed with 30 patients older than 60 years with a diagnosis of any cardiovascular disease. A parallel group ($n = 40$) age and gender matched was used as reference for the determined variables. Patients were treated with 200 ml ozone/oxygen mixture at 20 $\mu\text{g/mL}$ of ozone by rectal insufflation once a day during 15 days. **Results** Ozone improved the antioxidant status of patients by reducing biomarkers of protein and lipid oxidation and regulating the oxidant/prooxidant

balance. **Conclusion** Medical ozone treatment could be used to ameliorate the redox disruption in aged patients and as a complementary therapy in the treatment of cardiovascular diseases and its complications.

SCA2 predictive testing in Cuba: challenging concepts and protocol evolution.

Cruz-Mariño T, Vázquez-Mojena Y, Velázquez-Pérez L, González-Zaldívar Y, Aguilera-Rodríguez R, Velázquez-Santos M, et al. J Community Genet. 2015 Apr 19;6(2).

Spinocerebellar ataxia type 2 (SCA2) is a neurodegenerative disease caused by a CAG repeat expansion in the *ATXN2* gene. Cuba has the highest prevalence (6.57 cases/105 inhabitants) of SCA2 in the world. The existence of 753 affected individuals and 7173 relatives at risk prompted the development in 2001 of the first predictive testing program in the country. The medical records of over 1193 individuals, who requested the test within a 13-year period, were analyzed retrospectively. The presymptomatic and the prenatal tests had uptake rates of 43.4 and 23.9 %, respectively. Several ethical challenges resulted from this program. These include the following: (1) withdrawal due to the initial protocol's length; (2) the request to participate by 16 at-risk adolescents; (3) the decision made by ten out of 33 couples with a test-positive fetus to carry the pregnancy to term, leading to de facto predictive testing of minors; (4) the elevated frequency of the *ATXN2* gene large normal alleles (≥ 23 to 31 repeats) in the reference population. These issues have led to major changes in the guidelines of the predictive testing protocol: (1) the protocol length was shortened; (2) the inclusion criteria were expanded to reach at-risk adolescents with an interest in prenatal diagnosis; (3) interdisciplinary follow-up was offered to families in which test-positive fetuses were not aborted; (4) prenatal testing was made available to carriers of large normal alleles with ≥ 27 CAG repeats. The profiles of the participants were similar to those reported for other predictive testing programs for conditions like Huntington disease and familial adenomatous polyposis. The genetic counseling practices at the community level, the ample health education provided to the at-risk population, together with multidisciplinary and specialized attention to the affected families, are lessons from the Cuban experience that can be relevant for other international teams conducting predictive testing for other late-onset neurodegenerative disorders.

Semimechanistic model to characterize nonlinear pharmacokinetics of nimotuzumab in patients with advanced breast cancer.

Rodríguez L, Ramos M, Fernández E, Soriano JL, Peraire E, Castañeda G, et al. J Clin Pharmacol. 2015 Mar 11. DOI: 10.1002/jcph.496.

This study aimed (1) to develop a semimechanistic pharmacokinetic (PK) model for nimotuzumab in patients with advanced breast cancer and (2) to identify demographic, biochemical, and clinical predictive factors of the PK variability. Data from a phase 1 study were analyzed using the nonlinear mixed-effects approach (NONMEM). A target-mediated disposition model that included 2 open PK compartments, the monoclonal antibody (mAb)-target binding, and target and mAb-target complex turnovers best described the linear and nonlinear PK. Covariates had no influence on the PK parameters. The final parameter estimates were 19.93 L (steady-state volume), 0.0045-0.0172 L/h (range of total clearance values), 6.96 $\mu\text{g/mL}$ (steady-state binding constant), 5.50 h^{-1} (target degradation rate constant), 1.43 ($\mu\text{g/mL}$) $\cdot \text{h}^{-1}$ (complex formation rate), and 0.148 h^{-1} (complex internalization rate constant). The model described the effect of the mAb-target binding, and target and mAb-target complex turnovers on nimotuzumab PK. Simulations showed that doses above 200 mg maintained the 50% target occupancy during all of the treatment. This model can be very useful for knowing the dosing schedules required for efficacy and supports further investigation of the pharmacokinetic/pharmacodynamic relationships of nimotuzumab to improve its therapeutic use.

Specificity and sensitivity of visual evoked potentials in the diagnosis of schizophrenia: Rethinking VEPs.

González-Hernández JA, Pita-Alcorta C, Wolters CH, Padrón A, Finalé A, Galán-García L, et al. Schizophr Res. 2015 May 21. pii: S0920-9964(15)00264-9. DOI: 10.1016/j.schres.2015.05.007. [Epub ahead of print]

Alterations of the visual evoked potential (VEP) component P1 at the occipital region represent the most extended functional references of early visual dysfunctions in schizophrenia (SZ). However, P1 deficits are not reliable enough to be accepted as standard susceptibility markers for use in clinical psychiatry. We have previously reported a novel approach combining a standard checkerboard pattern-reversal stimulus, spectral resolution VEP, source detection techniques and statistical procedures which allowed the correct classification of all patients as SZ compared to controls. Here, we applied the same statistical approach but to a single surface VEP - in contrast to the complex EEG source analyses in our previous report. P1 and N1 amplitude differences among spectral resolution VEPs from a POz-F3 bipolar montage were computed for each component. The resulting F-values were then Z-transformed. Individual comparisons of each component of P1 and N1 showed that in 72% of patients, their individual Z-score deviated from the normal distribution of controls

for at least one of the two components. Cross validation against the distribution in the SZ-group improved the detection rate to 93%. In all, six patients were misclassified. Clinical validation yielded striking positive (78.13%) and negative (92.69%) predictive values. The here presented procedure offers a potential clinical screening method for increased susceptibility to SZ which should then be followed by high density electrode array and source detection analyses. The most important aspect of this work is represented by the fact that this diagnostic technique is low-cost and involves equipment that is feasible to use in typical community clinics.

State of malnutrition in Cuban hospitals; a needed update.

Santana Porbén S. Nutr Hosp. 2015;31(5):1900–9.

Rationale The Cuban Study of Hospital Malnutrition, as conducted during 1999 – 2001 with 1,905 patients assisted in 12 hospitals from 6 provinces of the country, revealed a hospital malnutrition rate of 41.2%. Having elapsed a decade after the first edition of the enquiry, update of this estimate is mandatory. **Objective** To update the state of hospital malnutrition in Cuba. **Material and method** Presence of malnutrition in 1,664 patients admitted to 12 hospitals from 8 provinces of the country between March 2012 and March 2014 was documented with the Subjective Global Assessment (SGA) survey of the nutritional status. The state of hospital processes of food and nutritional care was assessed by means of the Hospital Nutrition Survey (HNS). **Results** Current hospital malnutrition rate was 36.9% ($\Delta = +4.3\%$; $p < 0.05$). Completion rates of hospital exercises of nutritional assessment and of use of nutritional replenishment therapies were higher. **Conclusions** Having elapsed 10 years after the first edition of the ELAN CUBA Study, modest advances are seen in the identification and treatment of malnutrition in Cuban hospitals. It is perceived that formation and insertion of nutritionists verticalized in hospital care has contributed to the observed change. In addition, the activity of the Cuban Society of Clinical Nutrition and Metabolism in the advancement of the disciplines of nutritional therapy, artificial nutrition and metabolism might have served for a better recognition of the health problem posed by hospital malnutrition.

Weight Loss via Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis.

Vilar-Gómez E, Martínez-Pérez Y, Calzadilla-Bertot L, Torres-González A, Gra-Oramas B, González-Fabián L, et al. Gastroenterology. 2015 Apr 9. pii: S0016-5085(15)00496-5. DOI: 10.1053/j.gastro.2015.04.005. [Epub ahead of print]

Background and aims It is not clear how weight loss affects histologic features of liver in patients with nonalcoholic steatohepatitis (NASH). We examined the association between the magnitude of weight loss through lifestyle modifications and changes in histologic features of NASH. **Methods** We conducted a prospective study of 293 patients with histologically proven NASH who were encouraged to adopt recommended lifestyle changes to reduce their weight over 52 weeks, from June 2009 through May 2013, at a tertiary medical center in Havana, Cuba. Liver biopsies were collected when the study began and at week 52 of the diet, and analyzed histologically. **Results** Paired liver biopsies were available from 261 patients. Among 293 patients who underwent lifestyle changes for 52 weeks, 72 (25%) achieved resolution of steatohepatitis, 38 (47%) had reductions in NAFLD activity scores (NAS), and 56 (19%) had regression of fibrosis. At week 52, 88 subjects (30%) had lost 5% or more of their weight. Degree of weight loss was independently associated with improvements in all NASH-related histological parameters (odds ratios, 1.1-2.0; $P < .01$). A higher proportion of subjects with 5% weight loss or more had NASH resolution (51/88, 58%) and a 2-pt reduction in NAS (72/88, 82%) than subjects that lost less than 5% of their weight ($P < .001$). All patients who lost 10% of their weight or more had reductions NAS, 90% had resolution of NASH, and 45% had regression of fibrosis. All patients who lost 7%-10% of their weight and had few risk factors also had reduced NAS. In patients with baseline characteristics that included female sex, body mass index ≥ 35 , fasting glucose > 5.5 mmol/L, and many ballooned cells, NAS scores decreased significantly with weight reductions of 10% or more. **Conclusions** A greater extent of weight loss, induced by lifestyle changes, is associated with the level of improvement in histologic features of NASH. The highest rates of NAS reduction, NASH resolution, and fibrosis regression occurred in patients with weight losses of 10% or more.