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Neurological - Neurodegenerative diseases

Cod: W163

ANALYSIS OF SERUM 25-HYDROXYVITAMIN D LEVELS IN RELATION TO VDBP AND CYP27B1 ALELLIC VARIANTS IN MULTIPLE SCLEROSIS PATIENTS FROM SOUTH ITALY.

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Background: Multiple Sclerosis (MS) is a chronic demyelinating disease of central nervous system regarded as one of the most common causes of neurological disability in young adults. The exact etiology of MS is not yet known, although epidemiological data indicate that both genetic susceptibility and environmental exposure are involved. A poor vitamin D status has been proposed as the most attractive environmental factor. Several evidence have highlighted the importance of mutations in vitamin D-regulating genes for Vitamin D status. The purpose of our study was to assess genetic variants of VDBP (rs7041 and rs4588) and CYP27B1 (rs118204009, rs118204011 and rs118204012) in MS patients and in a control group.
Methods: a total of 192 subjects, including 100 MS patients and 92 healthy controls, were genotyped by polymerase chain reaction followed by restriction fragment length polymorphism analyses. Serum 25-hydroxyvitamin D levels were measured in MS patients and controls by high-performance liquid chromatography.
Results: 25(OH)D plasma levels were significantly higher in the control group when compared to MS patients (35±10 μg/l vs 21.5±7.3 μg/l, $p<0.05$). According to VDBP phenotype, we observed a trend to lower 25(OH)D plasma levels in MS patients who carried Gc2/Gc2 isoform (17.8±4.5 μg/l) and higher levels in Gc1f-1f (24.2±6.4 μg/l), even if this difference was not statistically significant. We did not observe any statically significant difference in the distribution of genotypic VDBP variants between study groups. We did not observe any wild type allele for CYP27B1 mutations analyzed both in MS patients and in the control group.
Conclusion: our findings do not support a role of an independent effect of the investigated vitamin D related gene variants, DBP and CYP27B1, in the risk of MS. Nevertheless, MS patients carriers of Gc2/Gc2 isoforms of VDBP could have lower 25(OH)D levels than carriers of other isoforms.

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Neurological - Neurodegenerative diseases

Cod: W164

EVALUATION OF PLASMA AGMATINE LEVEL AND ITS METABOLIC PATHWAY IN PATIENTS WITH BIPOLAR DISORDER DURING MANIC EPISODE AND REMISSION PERIOD

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Background: Agmatine is a cationic amine resulting from the decarboxylation of L-arginine. The reaction is catalyzed by the arginine decarboxylase which uses L-arginine as a substrate. Agmatine has neuroprotective, anti-inflammatory, anti-stress, anti-depressant properties. Agmatine also acts in the regulation of cognitive functions, and against morphine tolerance and dependence. However, no peer-reviewed studies have established that related to its role in mood disorders.
Methods: In this study, plasma agmatine levels were measured during manic episode and remission period in patients with bipolar disorder. We evaluated the relationship between levels of L-arginine and arginine decarboxylase in the agmatine synthesis pathway, and level of agmatinase that degrades agmatine. For this purpose, 30 healthy volunteers and 30 patients who meet Bipolar Disorder Manic Episode diagnostic criteria were included in the study. Additionally, the changes in the patient group between manic episode and remission period were examined.
Results: In the study, levels of agmatine and L-arginine were significantly increased than control group during manic episode ($p<0.01$). All parameters were increased during manic episode compared to remission period ($p<0.05$). Agmatinase was significantly decreased both during manic episode ($p<0.01$) and remission period ($p<0.05$) in comparison to the control group. Arginine decarboxylase levels did not show a significant difference between the groups ($p>0.05$).
Conclusions: Our results showed that agmatine levels of bipolar disorder patients were higher than healthy individiuals. Especially this increase is observed in manic episode. These results suggest that, increasing the agmatine level is a kind of neurochemical regulation which has anti stress effect to protect the brain in patients with Bipolar disorder. The results of this study indicate that there may be a relationship between bipolar disorder and agmatine and its metabolic pathway. Nonetheless, we believe more comprehensive studies are needed in order to reveal the role of agmatine in etiology of bipolar disorder.

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Neurological - Neurodegenerative diseases

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SERUM LEVELS OF NEURON-SPECIFIC ENOLASE, S100B AND C-REACTIVE PROTEIN IN SCHIZOPHRENIA

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BACKGROUND
Schizophrenia is associated with increased inflammation, including abnormal blood levels of C-reactive protein (CRP). Neuron-specific enolase (NSE) is used to investigate damage to neuronal structures. Glial placticity-inducing protein S100B is thought to be involved in pathogenesis of schizophrenia and also to be a marker of therapeutic response.
Our aim was to evaluate the value of serum biomarkers relevant for neuropsychiatric research and investigate their possible associations with clinical and cognitive features in patients with schizophrenia.
METHODS
We investigated serum samples from 91 patients with paranoid schizophrenia (34,6±9,9 years old, 51 males and 40 females). NSE, S100B and hs-CRP were determined using automated assays from Roche and Abbott. We also used PANSS, BACS and Rey–Osterrieth complex figure test.
RESULTS
Levels of NSE were 6.44±3.56 ng/ml, S100B – 0.044±0.021 μg/l (not exceeding normal values), CRP – 1.83±1.67 mg/l. 1/3 of patients had CRP levels between 3 and 10 mg/l, indicating the presence of systemic inflammation. Women had expectedly higher S100B concentrations (0.053±0.021 vs 0.037±0.019 μg/l). NSE levels were higher in treatment-resistant patients (N=34, 8.9±3.1 vs 5.1±3.0 ng/ml, $p<0.001$). Patients with family history of psychiatric disorders had significantly higher S100B levels (0.046±0.026 vs 0.038±0.015 μg/l, $p=0.026$). A positive correlation was found between protein levels for NSE and S100B and number of hospitalizations, but not with age or illness duration ($r=0.281$, $p=0.012$ and $r=0.289$, $p=0.010$, respectively). Thought disorganization was positively correlated with NSE and CRP levels ($r=0.347$, $p=0.026$; $r=0.433$, $p=0.017$). The latter was also higher in more hostile/agitated patients ($r=0.394$, $r=0.031$). Poorer short-term retention was associated with higher CRP levels ($r=-0.280$, $p=0.045$).
CONCLUSION
The severity of condition indicating poor therapeutic response (number of hospitalizations, several positive symptoms, treatment resistance) was correlated with laboratory parameters, though there was no significant increase in NSE or S100B concentrations. Patients with more impaired visuospatial short-term memory were characterized by higher levels of CRP. Our findings suggest that the activation of inflammatory response might be a pathogenic factor for the development of schizophrenia in some cases.
The study was supported by RSCF 14-50-00069 grant.

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Neurological - Neurodegenerative diseases

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S100B SERUM LEVELS: A BRAIN INJURY BIOMARKER IN DIFFERENT NEUROCRITICAL PATHOLOGIES

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Background: S100B serum concentration results altered after brain injury. In adittion, elevated serum levels of S100B have been reported to correlate with the degree of brain damage. The aim of this study is to evaluate the difference on S100B levels in serum from patients with different neurological disorders (aneurysmal subarachnoid hemorrhage (aSAH), severe traumatic brain injury (TBI) and intracerebral hemorrhage (ICH)) and a control group of healthy subjects.

Methods: 48 patients with aSAH, 199 patients with severe TBI and 21 patients with ICH admitted to the Neurosurgical Intensive Care Unit (UCI) of the Virgen del Rocío University Hospital were included in the study. The protocol was approved by the Hospital Institutional Review Board and written consent was obtained from the patients' relatives. The different protocols included the collection of a blood sample within the first 24 hours of the accident. Additionally, a group of 18 healthy subjects were volunteers to extract a blood sample. S100B serum levels were measured by automated electrochemiluminescence assay (ECLIA) (Cobas E602, Roche Diagnostics, Germany).

Results: We carried out a total of 286 measurements (aSAH: 18, TBI: 199, ICH: 21, control: 18). S100B values followed a non-normal distribution, hence we applied non-parametric statistical tests. Median values for serum S100B were:
–Control: 0.058 ng/mL (IQR: 0.029-0.075)
–SAH: 0.152 ng/mL (IQR: 0.084-0.211)
–TBI: 0.352 ng/mL (IQR: 0.215-0.722)
–ICH: 0.433 ng/mL (IQR: 0.316-1.056)

The Kruskal-wallis test stated that there was a significant difference on S100B values between all the studied groups ($p<0.001$). When applying the Mann-Whitney U test, we observed that there was a significant difference between the following groups:
–Control vs. SAH, $p<0.001$
–Control vs. TBI, $p<0.001$
–Control vs. ICH, $p<0.001$
–SAH vs. TBI, $p<0.001$
–SAH vs. ICH, $p<0.001$
Nevertheless, there was no significant difference between TBI and ICH S100B values ($p=0.114$).

Conclusion: Our results suggest that S100B serum levels are increased as a result of brain lesion. We found significant differences on S100B levels between controls and all the studied brain injury groups. Additionally, depending on the neurocritical pathology, S100B levels varied. The highest S100B concentrations were found in patients with ICH.

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Neurological - Neurodegenerative diseases

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THE EFFECT OF SEIZURES AND ANTIEPILECTIC DRUGS ON HORMONES

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BACKGROUND: Epilepsy is a neurological disorder. Endocrine changes are reported in epilepsy but the mechanisms of hormonal dysregulation are not clear. It is controversial whether the endocrine dysfunction in epilepsy patients is caused by the epilepsy itself, or the antiepileptic therapy, or both. In this study we investigate the effect of seizures and treatment with phenytoin or/and valproic acid, on prolactin (PRL) secretions and thyroid hormones (FT3, FT4 and TSH) secretions in male patients.

METHODS: Serum PRL levels and thyroid hormones levels were measured by electrochemiluminescence in 12 adult male epileptic patients, who were hospitalized with either tonic-clonic or/and partial seizures, in the neurologic clinic of AHEPA University Hospital. These parameters were also measured in 12 age and gender matched healthy subjects.

RESULTS: Male patients (415,17 ± 138,68 μIU/ml), as compared to male controls (188,5 ± 84,37 μIU/ml) had significantly higher levels of prolactin ($p < 0.001$). Interestingly, no significant difference was observed in thyroid hormones between patients and controls, as serum FT3 levels were in patients (4,38 ± 0,96 pmol/l), while in controls (4,03 ±1,20 pmol/l), serum FT4 levels were in patients (14,22 ± 2,74 pmol/l), while in controls (16,37 ±4,49 pmol/l) and serum TSH levels were in patients (2,81 ± 1,29 μIU/ml), while in controls (2,11 ± 1,15 μIU/ml), ($p=0,45$, $p=0,17$ and $p=0,89$, respectively).

CONCLUSIONS: The data in our study show that seizures of epilepsy and medication with antiepilectic drugs stimulate the secretion of PRL, as indicated by elevated serum prolactin levels in our patients. However epilepsy does not seem to affect thyroid function. We can also conclude that serum PRL levels could be a useful tool for the monitoring of the progression of seizures in epilepsy. Further investigation in this field is required.

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Neurological - Neurodegenerative diseases

Cod: W168

DO GENES CORRELATE WITH INTELLIGENCE ?

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BACKGROUND: Only 2% of the global population belong to those with elevated IQ. Research to date has had limited success in identifying DNA variants responsible for the heritability of intelligence. The aim of this study is to determine the effect of HTR2A/ rs1328674, FAAH/ rs324420, ANKK1/ rs1800497, SNAP25/ rs363050 and BDNF/ rs6265 on intelligence.
METHODS: 80 volunteers of Greek society of Mensa with known high IQ score (Figure reasoning test) and almost 900 volunteers of general population were examined. Following volunteer's informed consent undersigning, samples were de-identified and anonymized. DNA was isolated from epithelial cells collected from the oral cavity, using nucleic acid isolation columns. Genotypes were determined by real-time polymerase chain reaction using the Simple Probes commercial LightSnip kit. Logistic regression analysis was performed in all results.
RESULTS: rs1328674 G/G genotype is associated with High and very High IQ, with significant statistical differences ($p<0,05$) in all 5 different logistic regression models. Five genotype combinations of examined polymorphisms shown significant association with very high intelligence with $p<0,05$.
CONCLUSION: Preliminary data show that combined genotype determination of rs324420, rs1800497, rs363050 and rs6265 and rs1328674 polymorphisms have a close relationship to data derived from established IQ test. The rs1328674 polymorphism was statistically different between persons with very high IQ (>135) and the rest sample. High IQ score can be linked with the genetic background, as classified in this study.

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Neurological - Neurodegenerative diseases

Cod: W169

POPULATION-BASED ANALYSIS OF THE FREQUENCY OF HCRTR-2 GENE AND GNB3 GENE POLYMORHISMS AND INVESTIGATION OF THEIR CORRELATION WITH CLUSTER HEADACHES.

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Cluster headache (CH) is a type of primary neurovascular headache and there is an increased hereditary risk of CH.Genetic factors can affect the possibility of developing the disease.Studies found an association between CH and the polymorphism in the HCRTR2 gene,rs2653349.The less common mutated allele (A) seems to reduce the chance of developing the disease and the more common allele (G) increases the risk.The rs5443 polymorphism was associated with triptan treatment positive response and carriers of the mutated T allele who take triptans as treatment for cluster headaches were more likely to respond positively compared to C:C homozygotes.DNA from 1464 non related individuals was collected and analyzed, either from whole blood or buccal swabs.The frequency distribution of these gene polymorphisms was determined.The results for the polymorphism rs2653349 were G:G=77.8%,G:A=20.3% and A:A=1.9.The frequency of the wild-type allele was 92.3% and that of the mutated allele was 7.7%. Regarding rs5443 the results were C:C=44,8%,C:T=41.9% and T:T=13.3%.The frequency of the wild-type C allele was 70.0% and that of the mutated T allele was 30.0%.The OR of the male and female volunteers of the rs2653349 exhibited no statistically significant difference (p=0,4555) but for the rs5443 polymorphism we found a statistically significant difference(p=0,0292) between the genders.A comparison of the study population frequencies for the two polymorphisms versus other populations was also performed.The mutant allele (A) of rs2653349 appeared almost half as often in our study population (7.7%) as compared to the global population (12.1%) and much lower in the European population (18,4%).Also,we observed that the male homozygotes for the protective mutant allele are 2-fold more frequent than the female.The results also indicate that the investigated Greek population has great similarity to the European population regarding the allele distribution as well as the genotype distribution for the rs5443.The mutant allele (T) occurs in 30% of the Greek population and 32.8% in the European population.Based on our results we could assume that the pathophysiology of CH may be affected by multiple factors,however,the genotyping analysis of these polymorphisms may play a significant role in the overall treatment of patients suffering from cluster headaches.

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Neurological - Neurodegenerative diseases

Cod: W170

SELECTED PRO- AND ANTI-INFLAMMATORY CYTOKINE SERUM CONCENTRATIONS IN DIFFERENT CLINICAL FORMS OF MULTIPLE SCLEROSIS

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BACKGROUND:
Multiple sclerosis (MS) is an immune-mediated central nervous system disease characterized by inflammation, demyelination and axonal degeneration. The pathology of MS suggests an autoimmune cause involving cellular and humoral components of the immune system. Active MS lesions are characterized by T-cell and macrophage infiltration and the presence of immune mediators, including adhesion molecules, chemokines, and cytokines. Cytokines are proven mediators of immunological process in MS. The aim of this study was to delineate the serum cytokine profile in patients with MS and the controls and to determine in different clinical forms of MS.
METHODS:
This study involved 62 consecutive MS patients--28 patients with progressive MS and 34 patients with relapsing-remitting MS (RRMS). The control group consisted of 18, age and sex matched, non-immunological, neurological patients. The patients were evaluated using the Expanded Disability Status Scale (EDSS) and magnetic resonance imaging (MRI) with gadolinium. Serum samples for cytokine measurements were collected on admission. Plasma levels of proinflammatory T-helper (TH)1 (interferon (IFN)-γ) cytokines, peripheral monokines (IL-1beta, IL-6, TNF-alpha) and anti-inflammatory or down-regulatory TH2 cytokines (IL-4, IL-10) were determined by an enzyme-linked immunosorbent assay (ELISA) method.
RESULTS:
All patients with MS had significantly higher cytokine (IFN-γ, IL-1β, IL-6, TNF-α,IL-4,IL-10) concentrations compared with controls ($p < 0.001$). Increased IL-1beta, IFN-γ ($p=0.032$ and 0.041, respectively) and decreased IL-4, IL-10 ($p=0.038$ and 0.02, respectively) levels were found in progressive MS compared with RRMS. Patients with progressive MS with disease progression presented higher IL-1beta, TNF-alpha, IFN-γ and IL-10 levels than those without disease progression ($p < 0.05$). There was a significant inverse correlation between IL-10 levels and EDSS score in patients with progressive MS ($R = - 0.43$, $p < 0.05$).
CONCLUSION:
Profiling cytokines in multiple sclerosis may help to identify mechanisms involved in the pathogenesis of the disease, and, potentially, lead to new therapies directed at cytokines or their receptors. The level of IL-10 can serve as an additional diagnostic criterion for assessing the disability in patients with progressive MS.

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Neurological - Neurodegenerative diseases

Cod: W171

KEY PERFORMANCES OF LUMIPULSE® G ß-AMYLOID 1-42

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Background:
Today β-amyloid$_{(1-42)}$ peptide ($A\beta_{1-42}$) levels in cerebrospinal fluid (CSF) and amyloid imaging are well-accepted biomarkers representing Alzheimer's disease (AD) from the earliest stages on. Widespread use of these biomarkers in AD diagnosis requires highly precise, and accurate measurements. Analytical requirements and performance on CSF samples of the novel Lumipulse G β-Amyloid 1-42 assay (CLEIA) were verified and the key features are highlighted in this summary.
Methods:
The LUMIPULSE G instruments use single analyte, ready-to-use immunoreaction cartridges with a throughput of 60 and 120 tests/hour for the G600II and the G1200 instrument, respectively. Sequential immunoreaction steps are carried out at pre-determined intervals while the cartridge is transported through the system. Each cartridge generates quantitative results within approximately 30 minutes. The analytical assay performance was characterized according to the CLSI guidelines. Within the framework of the Alzheimer's Association QC program for CSF biomarkers, the inter-lab variability was evaluated. The assay was compared to the reference measurement procedure (RMP) for $A\beta_{1-42}$ (JCTLM ID: C11RMP9).
Results:
The obtained coefficients of variation (CSF and control samples) demonstrate a high level of precision, as may be expected for a highly standardized and automated assay platform. The low variability was confirmed in the inter-lab study. Analytical sensitivity was investigated on diluted CSF samples and the LoD and LoQ for the Lumipulse G β-Amyloid 1-42 assay were shown to be <15 pg/mL and <20 pg/mL, respectively. Linearity was shown across the clinical application range. High correlation with the RMP was obtained ($R > 0.95$).
Conclusions:
Automation, the mono test cartridge principle, short throughput times, and instrument flexibility are key attributes of the LUMIPULSE G instrument series making it the ideal platform to fulfill today's needs for rapid and accurate quantification of CSF biomarkers in both low and high throughput clinical laboratories. The novel CSF $A\beta_{1-42}$ assay on the LUMIPULSE G instruments shows good sensitivity, has a high level of precision, and is traceable to the standard reference measurement procedure.

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Neurological - Neurodegenerative diseases

Cod: W172

SERUM HEPCIDIN LEVELS IN ALZHEIMER'S DISEASE

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Background. Alzheimer's disease (AD) is characterized by deposition of amyloid plaques of amyloid-β chelating peptide with transition metal ions (Cu2+, Zn2+ # Fe3+). The binding of Cu2+ and Fe3+ leads to toxic chemical reactions; a change in the oxidation of two metals, that leads to H2O2 production in the presence of transition metals and finally gives toxic free OH• radicals.
Methods. 58 Alzheimer's disease patients were included. They were evaluated for serum iron, copper, zinc and hepcidin levels. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured as oxidative stress markers. Hepcidin, SOD and GPX were measured by ELISA methods. Serum Fe, Cu and Zn were quantified by AAS. The results form AD patients were compared to age and gender matched healthy controls. We used Pearson's correlation and Student's paired t-test for statistical analysis of established results.
Results. We found statistically significant elevated serum iron, copper and zinc results in AD patients (39.8 vs. 21.5 μmol/l; 35.6 vs. 18.4 μmol/l; 36.7 vs. 15.4 μmol/l; $P<0.01$). Hepcidin concentrations were increased in AD cases compared to controls (64.9 vs. 22.3 μg/l; $P<0.001$). SOD and GPX levels were decreased in Alzheimer's disease vs. normal values in healthy controls (8.9 vs. 19.8 μg/ml; 14.9 vs. 31.5 U/gHb; $P<0.001$). Two other AD cases, newly found showed increased SOD and GPX levels (28.9 μg/ml; 31.4 U/gHb; $P<0.005$).
Conclusions. The expected contribution from our study is practical introduction of quantification of serum hepcidin as a potential marker for early diagnosis of impaired iron homeostasis, leading trace element in the pathogenesis of neurodegenerative diseases.

We appreciate financial support of Medical University, Sofia, as this study is part of Project N° 5070/2016.

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Neurological - Neurodegenerative diseases

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PARKINSON'S DISEASE AND IRON HOMEOSTASIS, CONNECTION THROUGH HEPCIDIN

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Background. Parkinson's disease (PD) is characterized by the deposition of inclusion bodies (Lewy bodies) of α-synuclein in substantia nigra, which is ubiquitously expressed in the brain and mutations in this protein are presented in familial forms of AD. Dopamine as a neurotransmitter, and at the same time is a very good metal chelator and electron donor that define the conditions in vivo generation of toxic free radicals. It has a high propensity to coordinate with Cu2+ and Fe3+ and reduce metal primer Fenton generation of H2O2.
Methods. 39 Parkinson's disease patients were enrolled. They were quantified for serum iron, copper and hepcidin levels. Superoxide dismutase (SOD) and glutathione peroxidase (GPX) were measured as oxidative stress markers. Hepcidin, SOD and GPX were measured by ELISA methods. AAS was used for quantification of serum Fe and Cu. The results form PD patients were compared to age and gender matched healthy controls. Statistical analysis of established results was performed using Pearson's correlation and Student's paired t-test.
Results. We found statistically significant elevated serum iron and copper results in PD patients (41.5 vs. 20.4 μmol/l; 39.9 vs. 18.5 μmol/l; $P<0.01$). Hepcidin concentrations were increased in PD cases compared to controls (69.8 vs. 22.9 μg/l; $P<0.001$). SOD and GPX levels were decreased in Parkinson's disease vs. normal values in healthy controls (11.4 vs. 18.7 μg/ml; 15.6 vs. 30.9 U/gHb; $P<0.001$).
Conclusions. Expected contribution as a theoretical approach is clarification of oxidative stress as a result of impaired metabolism of trace elements in neurodegenerative diseases.

We appreciate financial support of Medical University, Sofia, as this study is part of Project N° 5070/2016.

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Neurological - Neurodegenerative diseases

Cod: W174

ASSESSMENT OF VASCULITIC NEUROPATHY WITH LP-PLA2 AND VEGF

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Background. Vascular endothelial growth factor (VEGF) is secreted from endothelial cells in response to hypoxia. Lipoprotein-associated phospholipase A2 (Lp-PLA2) induces angiogenesis and micro vascular hyperpermeability. It is produce mainly by monocytes, macrophages, lymphocytes, and mast cells and has been found to be upregulated in atherosclerotic lesions. Lp-PLA2 has proatherogenic properties by promoting modifcation of oxidized LDLs.
Methods. 9 patients with Poliarteriitis nodoza (PAN) were enrolled; 7 females and 2 males. They had clinical and neurological examination, EMG. They were evaluated for routine biochemical parameters, plus VEGF, Lp-PLA2 and hepcidin levels. Hepcidin, VEGF and Lp-PLA2 were quantified by ELISA methods. The results obtained from PAN patients were compared to age and gender matched healthy controls. Statistical analysis of established results was performed using Pearson's correlation and Student's paired t-test.
Results. We found statistically significant elevated serum VEGF and Lp-PLA2 results in PAN patients (85.4 pg/mL to 11.7 pg/mL; 22.9 ng/mL to 5.7 ng/mL; $P<0.005$). Hepcidin concentrations were increased in PAN cases compared to controls (71.4 µg/L to 20.7 µg/l; $P<0.001$). Plasma VEGF and Lp-PLA2 levels showed significant correlation to serum hepcidin concentration ($r=0.788$; $r=0.856$; $P<0.005$).
Conclusions. PAN is systemic disease characterized by necrotizing inflammation in the wall of various small to medium-sized arterial vessels. This leads to increased serum VEGF and Lp-PLA2 levels. On the other hand hypoxia elevates hepcidin concentration, deteriorating patients' conditions. Our results indicate that VEGF, Lp-PLA2 and hepcidin levels are associated with vasculitic neuropathy and may be used to predict this disease.

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Neurological - Neurodegenerative diseases

Cod: W175

CHARACTERISATION OF A PRIMARY CALIBRATOR FOR TAU QUANTIFICATION BY HIGH RESOLUTION MASS SPECTROMETRY.

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BACKGROUND

The concentration of tau in cerebrospinal fluid (CSF) is a well-established diagnostic biomarker of Alzheimer's disease. However, its clinical utility is hampered by the lack of standardisation of current immunoassays. To date, no reference method or material is available to ensure traceability of tau measurements to the International System of Units (SI) and their comparability over time. Within the European metrology project "NeuroMET", we aim to develop a reference method for tau quantification by isotope dilution, liquid chromatography mass spectrometry (ID-MS) to assess and improve accuracy of routine immunoassays.

METHODS

The high heterogeneity of tau in CSF complicates the definition of the measurand (total tau concentration). The chosen approach, supported within the IFCC working group of CSF proteins, consists in a bottom-up ID-MS method that targets tau peptide GAAPPGQK (amino acids 156-163), a non-modified peptide present in all tau isoforms. In order to produce unbiased results, the calibration approach relies on a well-defined recombinant tau calibrator. It has been characterised using a high resolution quadrupole-orbitrap mass spectrometer (MS) and quantified by amino acid analysis in a triple quadrupole MS.

RESULTS

Two methods for the characterisation of the recombinant tau calibrator have been investigated. The first one consists in amino acid analysis followed by the identification and correction of amino acid containing impurities. The second one is a bottom-up ID-MS method calibrated with tau proteolytic peptides. Impurity analysis for both tau and the proteolytic peptides has been performed by high resolution mass spectrometry. Preliminary results comparing the two characterisation approaches are presented, as well as the impact on the quantification of different CSF samples from Alzheimer's disease patients.

CONCLUSIONS

An ID-MS candidate reference method for total tau quantification in CSF is being developed. Two rigorous metrological procedures for the characterisation of the primary tau calibrator have been applied and compared. After validation, the method will be used to assign a SI-traceable tau concentration value to a commutable CSF pool and assess the feasibility of standardisation of routine immunoassays.

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Neurological - Neurodegenerative diseases

Cod: W176

THE EMPIR PROJECT: INNOVATIVE MEASUREMENTS FOR IMPROVED DIAGNOSIS AND MANAGEMENT OF NEURODEGENERATIVE DISEASES (NEUROMET)

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BACKGROUND:
Neurodegeneration is an incurable, debilitating process which presents a growing global challenge. Alzheimer's and Parkinson's disease are two of the most common neurodegenerative diseases (NDD). Both involve the build-up of specific proteins in the brain and subsequent neurodegeneration leading to physical and mental impairment and ultimately dementia. Here we present the overview of the EMPIR NeuroMET project, which brings together the diverse expertise of National Measurement Institutes, clinicians and academics, to overcome the specific measurement issues currently constraining clinical innovation in NDD diagnosis and treatment.
Here we provide an overview of the progress made in
1. developing improved magnetic resonance imaging (MRI) methods
2. understanding bias and uncertainty of immunoassays and digital PCR methods for neurodegenerative disease protein and miRNA biomarkers
3. development of reference methods for tau and alpha synuclein
4. improving the assessment of cognitive performance for early diagnosis of Alzheimer's disease

METHODS:
Liquid Chromatography Mass Spectrometry, LC-MS (triple quadrupole and quadrupole time of flight and quadrupole-orbitrap), immunoassays (single, multiplex and ultra-sensitive), digital PCR and magnetic resonance imaging and spectroscopy 7T (MRI and MRS) and modern psychometric analysis techniques

RESULTS:
Protocols for high resolution MRI of the whole brain and hippocampus and MRS of posterior cingulum were developed on healthy individuals and the preliminary results of the application of those protocols on a cohort formed by healthy, Alzheimer's disease and mild cognitive impairment individuals are presented.
An initial assessment of the uncertainty of immunoassays for quantification of Alzheimer's biomarkers in plasma including tau and neurofilament light chain was performed and applied on a number of platforms commercially available by using plasma samples from the Alzheimer's patient cohort.
A primary calibrator to be used for the analysis of α-synuclein and tau protein by LC-MS and for implementation of reference methods was developed
Preliminary results from psychometric analyses on cognitive performance measures are presented, identified key area for improvement.

CONCLUSIONS:
The early findings from EMPIR NeuroMET are promising, and the project is currently on schedule to deliver findings across the multiple sub-studies, and subsequent recommendations, by end of 2018.

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Neurological - Neurodegenerative diseases

Cod: W177

THE ITALIAN PROGRAM FOR STANDARDIZATION OF CEREBROSPINAL FLUID BIOMARKERS AS DIAGNOSTIC TOOL IN LABORATORY AND CLINICAL SETTINGS

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BACKGROUND. The redefinition of mild cognitive impairment and Alzheimer's disease (AD) has considerably modified criteria for diagnosis of neurodegenerative dementia, providing an increased level of certainty by evaluating the biomarkers of AD pathology (McKhann 2011). However, standardization of procedures is still lacking.
In Italy, scientific societies are synergistically moving with the aim to standardize the analysis of cerebrospinal fluid (CSF) biomarkers, amyloid ß 1-42, tau and phosphorylated tau in the clinical and laboratory practice.
In the first phase, the Italian Society of Clinical Biochemistry (SIBioC) and the Italian Society of Neurology and dementia (SINDem-ITALPLANED) have promoted a census of the laboratories and hospital which perform CSF analysis in a nationwide contest, focusing on critical issues as standardization of procedures, harmonization of ranges of normality and participation to quality control programs.
METHODS. Questionnaire was designed using SurveyMonkey and sent via email to the members of SIBioC, SINDem-ITALPLANED and to main Neurological Clinics all over Italy (n=1815).
RESULTS. We found an heterogeneous distribution of CSF laboratories along the territory, with several regions lacking of CSF biomarkers' evaluation (7/20). Some centralized laboratories (n=15) guarantee the biomarkers' analysis for neighbors memory clinics (n=15). Fifteen hospitals have an internal laboratory for CSF biomarkers. In sum, 40 neurological centers require CSF analyses. Both standardization and harmonization need a systematic organization. Only half of the laboratories (56.00%) participate in International Quality Control programs.
CONCLUSIONS. In conclusion, our data demonstrate that the use of CSF biomarkers is still limited in clinical practice and only a restricted number of patients receive an integrated clinico-biological diagnosis in Italy. In the next phase, the Scientific Societies will co-operate to define common national guidelines for both the standardization of CSF analysis in laboratory and the appropriate use of diagnostic tool in clinical setting, with the aim to allow a better harmonization of the CSF biomarkers along the territory.

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Neurological - Neurodegenerative diseases

Cod: W178

OLIGOCLONAL BAND ANALYSIS VS THE KAPPA AND LAMBDA FLCS ASSAY FOR THE DIAGNOSIS OF MULTIPLE SCLEROSIS

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Management of multiple sclerosis (MS) is facing novel scenarios especially concerning the diagnostic procedure. Until 2010, oligoclonal band (OCB) analysis by mean of isoelectric focusing was considered the Gold Standard technique for diagnostic confirmation. Indeed, the technique is cumbersome and in the evaluation of samples yielding dubious OCB responses (i.e. a single band) reveals interpretative limitations. In our experience, about 15% of total OCBs yielded dubious responses. Recently, free light chains (FLCs) has been suggested as potential diagnostic value in the context of MS. Thus, the k and λ FLCs assay for intrathecal FLC quantification is emerging as a promising tool for supporting MS diagnosis.
We hereby describe 2 patients who underwent OCB analysis and FLCs quantification in cerebrospinal fluid (CSF) and serum using Freelite™ (The Binding Site Ltd, UK), a turbidimetric assay.
A 43 years old man, affected by visual impairment and left homonym hemianopia, showed a negative IgG index, positive CSF/serum albumin ratio (6.1) with a single supernumerary OCB in CSF analysis. CSF and serum k and λ FLCs were within the reference range while the kFLC index was positive (13,6). Visual evoked potentials and MRI suggested retrobulbar optic neuritis indicative of clinically isolated syndrome (CIS).
A 55 years old woman affected by visual impairment of left eye lasting one week and mild retro-orbital pain presented to the emergency department. CSF analysis yielded negative CSF/serum albumin, negative IgG index and a single supernumerary OCB. Despite the extra OCB (alike the previous patient), FLC quantifications were out of range (CSFFLCk =1.90 , CSFFLCλ=0.45). FLC index was highly abnormal (56,3). MRI, revealing optic neuritis and brain parenchymal signal abnormalities, confirmed the suspicion of MS.
The FLCs CSF test may be a valid alternative to support MS diagnostic protocols, especially in the event of dubious or unclear OCB (with negative microbiological results) and in order to confirm or reject all suspicions. FLCs assay may be easily performed (as all automated tests) as initial screening upon sample request submission. Further studies are required to confirm its use in this context.

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Neurological - Neurodegenerative diseases

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OXIDATIVE STRESS IN AlCl3-INDUCED STRIATAL NEUROTOXICITY

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Background: Brain exposure to aluminium is associated with neuronal and astroglial cell damage in the selective vulnerable brain regions. Aluminium chloride (AlCl3) is neurotoxic and has been shown to induce experimental neurodegeneration and can promotes an oxidative stress in the brain tissue. The objectives of the study were to examine the effects of intrahippocampal AlCl3 injection on superoxide anion radical (O2•-), malondialdehyde (MDA) concentration, reduced glutathione (GSH) content, as well as superoxide dismutase (SOD) activity in the ipsi- and contralateral striatum of Wistar rats. Methods: A single dose of AlCl3 (3.7 mg/kg b.w.) was applied to adult male Wistar rats unilaterally in the CA1 sector of hippocampus, in the volume of 0.01 ml, using stereotaxic instrument for small animals. Control rats were treated with 0.9% saline solution in the same manner. Animals were sacrificed 10 min and 3 days after the treatment, heads were immediately frozen in liquid nitrogen and both hemispheres of the striatum were removed. Results: Markedly increased production of O2•- in the striatum bilaterally after 10 min (ipsi- for 41.5%, contra- for 39%) and contralaterally by 20.5% after 3 days of the AlCl3 injection, along with elevated lipid peroxidation bilaterally in the striatum (ipsi- for 72%, contra- for 74% after 10 min and ipsi- for 26.5%, contra- for 55% after 3 days) altogether contributed to AlCl3 neurotoxicity. Increased GSH content (ipsi- for 66%, contra- for 92% and ipsi- for 32%, contra- for 56% within 10 min and 3 days, respectively), and considerably low activity of SOD bilaterally after 10 min (ipsi- for 36%, contra- for 27.5%) and contralaterally by 33% after 3 days of AlCl3 application were measured in the striatum compared to control rats. Also, the results suggest a significant positive Spearman correlation between MDA concentration and GSH content in the ipsilateral striatum after 10 min of AlCl3 injection ($r = 0.9429$, $p < 0.01$). Conclusion: The biochemical changes observed in neuronal tissue have shown that aluminium acts as a pro-oxidant. Our findings confirmed that intrahippocampal AlCl3 injection spread temporally and spatially to the striatum and indicate that AlCl3 toxicity promotes the development of oxidative stress in this brain structure.

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MGLU2 MGLU3 EXPRESSION LEVELS IN RAT BRAIN AREAS PREFRONTAL CORTEX AND STRIATUM AT DIFFERENT GLUTAMATE DOSES IN SCHIZOPHRENIA MODEL

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Glutamate and glutamate receptors which are widely to serve in the brain synapses , suggesting that the brain main glutamate pathways in the etiology of many diseases hosting psychosis. Etiology of many diseases such as mania, depression, cognitive disorders and Alzheimer's disease develop psychosis. However, although a narrower range as a group of diseases using the diagnostic criteria of psychosis, schizophrenia is one of the diseases in this scale.
In this research, we planned a ketamine induced NMDA antagonism in Wistar rats, in order to examine the role of metabotropic glutamatergic receptors modulation and expression in schizophrenia pathology. It is stated that ketamine which is an anesthetic should be treated in subanesthetic dose to be able to used in schizophrenia modeling. For this reason; ketamine was treated daily in 30mg/kg for 5 days. Volume of injections was not exceed 1ml/kg and implamented as intraperitoneale .On the day when the injections were completed, brain tissues of the animals were immediately removed under phenobarbitale (50mg/kg) anesthesia and these tissues were maintained under -80°C until the laboratory research time. Receptors expression levels were detected by western blotting method in kontrol and ketamine groups and also in the experiment groups generated by different L-glulutamate doses. We evaluated the statistical significance of the data, SPSS 20 program.
According to the statistical results we obtained in our study; metabotropic glutamate receptor levels (mGlu2 , mGlu3) in both brain regions prefrontal cortex and striatum were decreased in high concentrations of L-glutamate, whereas no significant changes in the metabotropic glutamate receptor expressions in lower concentrations of L-glutamate.
Decreasing expression level of metabotropic glutamate receptor at high concentrations of L-glutamate suggests that if intracellular calcium concentration state is primary releated with autoreceptor function.

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Neurological - Neurodegenerative diseases

Cod: W181

ALPHA-SYNUCLEIN PATHOLOGY TRANSMISSION? EMERGING ROLES OF THE KLK6 PROTEASE FROM NEW IN VITRO AND IN VIVO MODELS

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KLK6 is a serine protease, which is highly expressed in the human nervous system. In synucleinopathies, including Parkinson disease, the levels of KLK6 in CSF correlate inversely with the levels of α-synuclein. Multiple evidences converge that specific species of the extracellular α-synuclein are implicated in synucleinopathies, nonetheless, the underlying mechanisms remain largely unexplored. Deciphering the molecular mechanisms that ensure normal turnover of naturally secreted α-synuclein may provide novel insights into our understanding of the disease and potential new targets for treatment. A state-of-the art hypothesis is under investigation according to which disease progression is associated with a "prion-like" propagation of α-synuclein fibrils. We showed recently that KLK6 can degrade the extracellular α-synuclein directly and indirectly via a proteolytic cascade that involves downstream proteases activated by KLK6 (Ximerakis et al. FASEB J, 2014). Proteomics identified α-synuclein among the top KLK6 substrates. A TAILS (Terminal Amine Isotopic Labeling of Substrates) degradomics approach revealed MMP2 and ADAMTS19 as the proteases activated by KLK6 in a neuronal environment (Pampalakis et al. Oncotarget, 2016). Using new models we provide first-time evidence that the naturally secreted KLK6 can readily cleave synthetic α-synuclein fibrils that are neurotoxic and have the ability for cell-to-cell transmission. We have generated Klk6–/– mice (collaboration with Prof. Andras Nagy, University of Toronto, Canada) and assessed these by standard behavioral tests. Klk6–/– mice exhibited reduced strength in the grip assay, consistent with motor defects, which could be reminiscent of Parkinson-like symptoms. Intriguingly, increased levels of high molecular weight α-synuclein species were found in the cortex of Klk6–/– animals at 3 months of age, while Klk6-deficient cortical neurons had increased ability for fibrillar α-synuclein uptake. We have constructed adenoviral vectors for KLK6 delivery and demonstrated that the levels of extracellular α-synuclein can be regulated by neuronally secreted KLK6. These findings open up the possibility to exploit KLK6 as a putative pharmaceutical protein for synucleopathies and may represent a novel pharmacological approach for therapeutic intervention.

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Neurological - Neurodegenerative diseases

Cod: W182

CEREBROSPINAL FLUID PROTEINS IN DIFFERENTIAL DIAGNOSIS OF CNS DISEASES

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The differential diagnosis between inflammatory and non-inflammatory diseases of CNS includes CSF quantitative measurements of immunoglobulin and albumin concentrations and their comparison to the serum. For that purpose we estimated the concentrations of albumin and immunoglobulin G both in CSF and serum of patients with inflammatory and non-inflammatory diseases of CNS. In parallel, these parameters were determined in the control group composed of patients having the values of investigated parameters within reference ranges. The obtained data were statistically evaluated by nonparametric Mann-Whitney test. The values of calculated CSF/albumin ratio have shown significant differences between the both patient groups and the control one ($p < 0.001$). It proves that in each examined group the blood brain barrier is damaged. For the assessment of intrathecal IgG synthesis we have calculated both IgG/albumin index and Schuller index. The comparison of the values obtained for patients with non-inflammatory diseases to the control one, revealed no statistically significant differences ($p > 0.05$). On the contrary, significant difference have been observed between the group with inflammatory diseases and the control ($p < 0.05$), proving the existence of IgG intrathecal synthesis. Values of both indexes were much higher in patients with inflammatory than non-inflammatory diseases, meaning that they can be used in differential diagnosis.