



RBSC2013

REGIONAL EUROPEAN BIOMEDICAL
LABORATORY SCIENCE CONGRESS

Under the
Patronage of



Regional European Congress of Biomedical Laboratory Science & the 4th Greek Medical Laboratory Technologists Conference "Technical Advances and Current Practices"

December, 5-7, 2013

Athens, Greece - TITANIA Hotel



**Book of Abstracts &
Congress Programme**



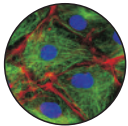
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Ο αξιόπιστος συνεργάτης στις καινοτόμες επιστημονικές λύσεις



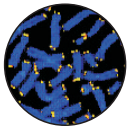
Μοριακή Διαγνωστική (Molecular Diagnostics)

- Haematology
- Molecular Microbiology
- Human Genetics



Μοριακή και Κυτταρική Βιολογία (Molecular & Cell Biology)

- Nucleic Acid Purification and Analysis
- Nucleic Acid Amplification and Expression Profiling (PCR, RT-PCR, Real-Time PCR reagents)
- Cloning
- Proteins, Expression, Isolation and Analysis
- Cell Biology
- Cell Culture
- Cell and Tissue Analysis
- Gene Regulation (RNAi, Epigenetics)
- Gene Expression Profiling and Genotyping
- Radiometric Detection
- Multilabeling Detection
- Automatic Caryotyping and Fish Analysis



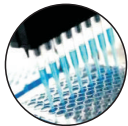
Γεωμική - Πρωτεομική (Genomics - Proteomics)

- PCR Amplification, Real Time PCR
- Gel Documentation & Analysis
- Lab Automation



Ιατροδικαστικά - Εγκληματολογία (Forensics)

- Human Identification
- Chemical Analysis
- Lab Automation



Αναλυτικές Μετρήσεις (Analytical Measurements)

- Environmental Measurements
- Food Flavors and Agricultural Analysis
- Petrochemical Analysis and Biodiesel
- Material Characterization
- Pharmaceutical Manufacturing and Quality Control



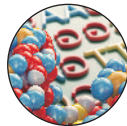
Μικροσκοπία και Απεικονιστική (Microscopy and Imaging)

- Light Microscopes for Biology & Medical Sciences
- Educational Microscopes
- Laser Microdissection Systems
- Confocal microscopy
- Material Microscopy
- Imaging Microscopy Systems / Digital Microscopy Cameras



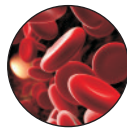
Γενικές Εργαστηριακές Εφαρμογές (General Laboratory Applications)

- Sample Preservation
- Centrifugation
- Incubation
- User & Sample Protection Systems
- Liquid Handling
- Basic Laboratory Equipment
- Laboratory Consumables, Reagents & Chemicals
- Cleanroom & Critical Environment Equipment
- Autoclave & Sterilization Equipment



In Vitro Διαγνωστικά

- Βιοχημεία
- Ανοσολογία
- Αιμόσταση
- Αέρια αίματος
- Αιματολογία
- Ανάλυση ούρων
- HPLC για HbA1c και αιμοσφαιρινοπάθειες
- LIS Software



Υλικά Αιμοδοσίας

- Ασκοί αίματος
- Φίλτρα λευκαφαίρεσης
- Συστήματα αδρανοποίησης παθογόνων στα παράγωγα αίματος
- Ασκοί συλλογής ομφαλοπλακουντιακού αίματος
- Αντιδραστήρια ανοσοαιματολογίας
- Εξοπλισμός αιμοδοσίας
(κλίβες, αναδευτήρες, ψυγεία, συγκολλητές, πρέσες κ.λ.π.)

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Welcome Letter

Dear Colleagues and Friends,

On behalf of the Scientific and Organizing Committee, it gives me great pleasure, to invite you to the Regional European Congress of Biomedical Laboratory Science & the 4th Greek Medical Laboratory Technologists Conference "**Technical Advances and current practices**" (<http://ebsc2013.com/>), under the auspices of EPBS, on 5-7 December 2013, in Athens, Greece.



Our primary goal is to develop the profession of Biomedical Scientists and emphasize our role in the health care system, where Biomedical laboratory Science plays an important role throughout the world, providing excellent clinical laboratory service for the wellbeing of the patients.

This Biomedical congress promises to be a memorable professional and scientific experience and is expected to draw hundreds of highly qualified participants from around the world, to discuss and share the latest knowledge in biomedical laboratory science: Pathologists, Medical Scientists, Medical Laboratory Technologists and Technicians, researchers, as well as Laboratory Suppliers from all fields of Laboratory Medicine.

Featuring distinguished speakers the high-quality scientific program will broaden perspectives, by exploring innovations in the latest diagnostic and research technology and by promoting modern scientific and technological developments in Biomedical Science, in the following scientific fields: Haematology-Blood Bank, Immunology, Microbiology, Biochemistry-Clinical Chemistry, Cytology, Molecular Biology-Genetics, Pathological Anatomy, Certification and Accreditation of Laboratories, Bio safety, Bioinformatics and Ethics in the Biomedical Laboratory.

Furthermore, our scientific programme will include information about prospects and career opportunities, as well as the Certification for the Biomedical Scientists, in Europe and globally.

We also look forward to welcoming you to Athens, known for its history. Despite the tight schedule and besides all the hard work during our meetings, there will be some spare time for social events to experience Athens with its rich culture and heritage, offering participants the opportunity to enjoy the sights of our fascinating city.

Yours faithfully
Dionysis Vourtsis, MT
Congress Chairman



PETIE (PanHellenic Association of Medical Laboratories Technologists) is the Scientific and Professional association of Medical Technologists in Greece and It counts 1450 members. The most significant event in the history of the association is the issue of the Professional rights (163/14.6.1996).

Main Objects:

- The protection and progress of the scientific - technological level of Medical Technologists, as well as their ethical and financial development.
- The promotion and establishment of MT's scientific - occupational rights.
- The participation to relevant organizations in Greece or abroad, which aims the progress of Biomedical Science, their broadest possible development and the promotion of common goals.
- The use of any means (studies, lectures, seminars, congresses, publications etc), for the methodical and responsible information of the people, members and the authorities on specific issues on Biomedical Science.
- The qualitative upgrade of the medical care in Greece, which should correspond not only to the needs, but also to the expectations of the people.

PETIE is a member of:

- **IFBLS**, International Federation of Biomedical Laboratory Science
- **EPBS**, European Association for Professions in Biomedical Science
- **ASCP** American Society of Clinical Pathology (Collaborating Society)

Scientific Committee

Presidents

Marie Culliton, MSc, MBA, FAMLS, EPBS President, Ireland – President

Anastasios G. Kriebardis, MSc, PhD, Greece

Vice Presidents

Fernando Mendes, MSc, EPBS General Secretary, Portugal – Vice President

Petros Karkalousos, MSc, PhD, Greece

Members

Apostolos Beloukas, MSc, PhD, Greece

Anne Berndt, MSc, EPBS Management Body, Sweden

Vassilios Birtsas, BSc, MSc, National Organisation of Medicines, Greece

Nikolaos Bournousouzis, BSc, MSc, Greece

Sonia Daadoucha Perroud, EPBS Management Body, Switzerland

Vincent S. Gallicchio, PhD, FACS, FRSA, FASAHP, IFBLS Past President, USA

Barbara Kappeller, EPBS Student Facilitator, Austria

George Albert Karikas, PhD, Greece

Kyoko Komatsu, PhD, IFBLS President, Japan

Athena Mavridou, MSc, PhD, Greece

Stella Mitka, PhD, MD, Greece

Anneke Geurts-Moespot, EPBS Management Body, Holland

Agelos Papaioannou, PhD, Greece

Petros Papalexis, BSc, MSc, Greece

Nikolaos Parisis, MSc, PhD, France

Panagiotis Plageras, PhD, Greece

Gabriele Sander, Austria

Mirjana Stupnisek, MSc, PhD, IFBLS Board of Directors, Croatia

Brit Valaas Viddal, Chair NITO – Norwegian Institute of Biomedical Science, Norway

Myrto P. Zacharof, M. Phil, PhD, AMICHEM, UK

Invited Speakers

Marie Culliton, MSc, MBA, FAMLS, EPBS President, Ireland

Thomas Kakouli-Duarte, PhD, Ireland

Fernando Mendes, MSc, EPBS General Secretary, Portugal

Mirjana Stupnisek, PhD, MLT, Croatia

Kyoko Komatsu, IFBLS President, Japan

Marie Nora Roald, Norway

Tone Nygard Wedø, Norway

Alba Marzo, PhD, Italy

Angelo D'Allessandro, PhD, Italy

Cesare Duranti, Italy

Auspices

EPBS - European Association for Professions in Biomedical Science

PEIB – PanHellenic Association of Medical BioPathology

Greek Health Ministry

Organizing Committee

President

Dionysis Vourtsis

Members

Petros Papalexis

Panagiotis Drosos

Ioannis Zannopoulos

Giorgos Kanterakis

Ioannis Mpithimitris

Olga Giannisi

Nikolaos Sxinas

Giorgos Diamantopoulos

Efi Pavlou

Eleni Papageorgiou

Ioannis Karvounis

Gregory Ananiadis

Andreas Xristoforidis

Dimitris Vasdekis

Apostolos Apostolidis

Konstantinos Davrados

Maria Koutsoliakou

Panagiotis Patoulas

Eleni Limperi

Anna Pyrovolaki

Antigoni Pouli

Ioannis Papadopoulos

Katerina Danezi

Sofia Mpinioti

Xristina Karanasiou

Armine (Maria) Pogosian

Martha Kyriakidou

Welcome

With Thanks to our Sponsors and Supporters



General Information

Congress Venue

Titania Hotel

Panepistimiou 52

Athens 106 78, Greece

Tel: +30 210 33 26000

Fax: +30 210 33 00700

Congress Dates

Thursday, 5th December – Saturday, 7th December, 2013

Language

The official language of the Congress is English. All abstract submissions and presentations are to be in English.

It will be also a Greek session with the Oral abstract presentations of the 4th PanHellenic Medical Laboratory Technologists Conference.

Airports and Transportation

Athens International Airport 'Eleftherios Venizelos' is located approximately 27 km from the city. You can get to/from the airport by taxi, bus or metro.

Taxi: The journey time by taxi is approximately 35 minutes and the flat fare to the city center is €35 during the daytime (05:00-24:00) and €45 during the nighttime (00:00-05:00).

Bus: The 24-hour Express bus X95 will take you directly to Syntagma square in the city center. The bus terminal is located in front of the Arrivals Terminal. Journey time is around 1 hour.

Metro: Take Metro Line 3 to Syntagma in the city center

Local Climate

The temperature during December ranges between 15°C (59°F) during the day to around 8°C (46°F) at night. Daylight is around 10 hours.

Congress Dress Code

Informal for all events.

Banking and Exchange

The Euro is (currently) the official currency of Greece. For a small transaction fee you can withdraw Euro's from the many ATM machines located around the city. We recommend you use cash when dealing with local merchants as you will get a better deal than if you use your debit or credit cards. Greek banks operate from Monday –Thursday from 8:00am to 2:30pm and Friday from 8am to 2pm. Make sure to bring your passport. You can also exchange money at large hotels and travel agents but note that there will be extra fees and commission.

Visas

All visitors are required to have a valid passport, and for some countries, an entry visa is required. For more information please contact your nearest Greek Consulate or local travel agency well in advance. It is the responsibility of the participant to obtain a visa if required.

Letter of Invitation

Official letters of invitation designed to assist with obtaining an entry visa can be requested from the Congress Secretariat, by e-mail or by post. Please note that such letters do not represent a commitment on the part of the Organising Committee or Congress to provide any financial assistance.

Liability and Insurance

The organiser is not able to take any responsibility whatsoever for injury or damage involving persons and property during the Congress. Participants are advised to take out their own personal travel and health insurance for their trip.

Registration & Accommodation

All participants must submit a completed registration form. Hotel accommodation at special Congress rates will be available to participants on the Congress website. Accommodation will open shortly.

Congress Secretariat

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Paragon Group

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**Regional European Congress of Biomedical
Laboratory Science & the 4th Greek Medical
Laboratory Technologists Conference**

"Technical Advances and current practices"

5-7 December 2013, Athens, Greece

Programme

www.ebsc2013.com

www.petie.gr

Thursday, 5 December 2013

Round Tables, Lectures and Opening Ceremony

08:00-09:30 Opening Registration

9:30-11:30, Laboratory Animals Round Table

Using animals in Biomedical Research

Chair: Dr. Nikolaos Kostomitsopoulos

Lectures

- 09:30** **New legal framework on the use of animals in Biomedical Research,**
Dr. Chrysa Voyiatzaki, Greece
- 10:00** **Education and training guidelines for those involved in animal experimentation,**
Prof. Ismene Dontas, Greece
- 10:30** **Early recognition and classification of pain,** Irene Symeon, Greece
- 11:00** **Ethics in animal-based research,** Dr. Nikolaos Kostomitsopoulos, Greece

11:30-12:00 Coffee break

12:00-13:30, Laboratory Animals Round Table

Experimental animal models for designing human vaccines: from Edward Jenner to tomorrow's vaccines

Chair: Eleni Dotsika, DVM, PhD

Lectures

- 12:00** **Advances in antigen selection for developing effective vaccines,**
Maria Agallou, Biologist, PhD, Greece
- 12:30** **Vaccine adjuvants as tools for designing innovative vaccines,**
Olga Koutsoni, Biologist, Greece
- 13:00** **Novel particulate vaccines utilizing nanotechnology,**
Maritsa Margaroni, Biologist, MSc, Greece

13:30-14:30 Break

14.30-16:30, Microbiology Round Table

Infectious Diseases Causing Public Health Issues in Greece

Chair: Dimitris Fourkas, Prof. Joseph Papaparaskevas

Lectures

- 14:30** **HIV:** Prof. Mina Psychogiou, Greece
- 15:00** **West Nile virus disease: Surveillance data and public health measures**
Annita Vakali, MSc, Greece
- 15:30** **Malaria:** Dr. Evangelia-Theophano Piperaki MD, Greece
- 16:00** **Rabies:** George Rigakos, MD, Greece

16:30-17:00 Coffee break

Programme

17:00-19:00 Biomedical Scientist in Europe

Chair: Prof. George Karikas, Prof. Athena Mavridou, Dionysis Vourtsis

17:00 Use of Ethical Reflection Model to Develop Ethical Competence, Marie Nora Roald, Norway

17:25 Biomedical Science Education across Europe – enhancing the Profession, changing the Laboratory's, Fernando Mendes, EPBS General Secretary, Portugal

17:45 The role of Biomedical Laboratory Scientists (BLS) in Greece, Dimitrios Togkas, Greece

18:00 Education of Biomedical Laboratory Technologists in Croatia, Mirjana Stupnisek, PhD, MLT, Croatia

18:20 Distinctive Professional Competencies of Biomedical Laboratory Technologist in Italy, Alba Marzo, Italy

18:35 Raising the profile of Biomedical Scientists in Europe, Marie Culliton, EPBS President of, Ireland

19:00-19:30 Coffee break

19:30-20:30 Opening Ceremony

Welcome and Opening Ceremony

Official Greetings:

President of EPBS: Marie Culliton, Ireland

General Secretary of EPBS: Fernando Mendes, Portugal

President of IFBLS: Kyoko Komatsu, Japan

President of PETIE: Dionysis Vourtsis, Greece

20:00, Opening lecture

Chair: Professor of Clinical Chemistry from University of Athens

Kinetics and genomic approaches to optimize pharmacotherapy.

The role of Biomedical Laboratory Scientist: Prof. Giorgos Karikas, Greece

Friday, 6 December 2013

Scientific Oral & Poster Abstract Presentations

HALL -A

09:00-11:00, Haematology Oral Presentations

Chair: Prof. Anastasios Kriebardis, Prof. Maria Benetikou

09:00 Informatic Crossmatch in Transfusion Medicine, Fernando Mendes, Portugal

09:20 Cytotoxic Effects of ionizing Radiation in Cancer Cell Lines of Small Cell Lung Cancer and Large Diffuse B cell Lymphoma – A Therapeutic tool, Fernando Mendes, Portugal

09:40 Erythrocyte Characteristics of Young Female Blood Donors, Vassilios Balomenos, Greece

10:00 Plasma Microparticles: A New Challenge on the Way of Centrifuging Blood in Coagulation Testing, Dimitrios Vasdekis, Greece

Haematology Lecture

10:30 Differential count. “Will the choice of method for making a smear have any influence on the DIFF result? Morphology, how to maintain the competence?”,
Tone Nygard Wedø, Norway

11:00-11:30 Coffee break

HALL -A

11:30-12:30 Informatics/ Education/Clinical chemistry Oral Presentations

Chair: Mirjana Stupnisek PhD, Prof. Petros Karkalousos

11:30 Informatics Procedure in Laboratory Management: Implementation of an Online Course to Maintain Expertise, Cesare Duranti, Italy

11:45 Predictors of Success for MLS Students, Vicki Freeman, USA

12:00 Comparative Study of Serum CA 19-9 and CEA Levels, Measured by Two Immunometric Assays (ELISA and FIA) in Different Diagnosis of Malignant Pathologies,
Ridvana Mediu, Albania

12:15 Prevalence of Anti-Insulin, Anti-IA2, Anti-GAD, Anti-IR Auto-antibodies and Anti-Neu5Gc Antibodies in Diabetic Type I And II Patients, Phaedra Eleftheriou, Greece

HALL -A

12:30-14:00, Laboratory Haematology Round Table

Modern laboratory investigation of Acute Myeloid Leukemia

Chair: George Androutsos, Efi Pavlou

12:30 Cytomorphology and cytochemistry in Acute Myeloid Leukemia,
Eirini Kritikou-Griva, PhD, MD, Greece

13:00 Flow cytometry in diagnosis and minimal residual disease assessment in Acute Myeloid Leukemia, Dr. Giorgos Paterakis, PhD, MD, Greece

13:30 The genetic background of Acute Myeloid Leukemia, Papadimitriou SI, MD, Greece

Programme

14:00-15:00 Break

HALL -A

15:00-16:00 Blood Banks Lectures Round Table

Recent data on Blood Safety. Storage and viruses

Chair: Prof. Anastasios Kriebardis, Dr. Marianna H. Antonelou

Lectures

- 15:00 Red blood cell storage: the Integrated Omics perspective,**
Dr. Angelo D' Alessandro, Italy
- 15:20 Emerging viral infections: A potential threat for blood safety,**
Dr. Mary Adamopoulou, Greece
- 15:40 Recent findings in HIV Cure research towards. HIV/AIDS pandemic evolution,**
Dr. Apostolos Beloukas, UK

HALL -A

16:00-17:30, Doping control Round Table

Procedures for a valid and useful result

Chair: Georgios Mavrotas

Lectures:

- 16:00 Doping control Test Distribution Planning:** Efstathios Cookeas, Greece
- 16:20 The sample collection process in Doping Controls:** Nikolaos Bournousouzis, Greece
- 16:40 Recent Advances in Doping Analysis:** Dr. Manolis Lyris, Greece
- 17:00 Doping control Results Management:** Efstathios Cookeas, Greece

17:30-18:30 Coffee break

HALL A

18:30-20:00, Applied Microscopy Techniques Round Table

Chair: Dr. Dimosthenis Stamopoulos

- 18:30 Biopsy of peripheral blood smears with advanced microscopes: focusing on the anemia observed in hemodialysis patients,** E. Manios, Greece
- 18:50 Evaluation of Protein and Cell Arrays Created on Micro- or Nanostructured Substrates by Fluorescence Microscopy,** Panagiota Petrou, Greece
- 19:10 Cytogenetics in patients with de novo and secondary acute myeloid leukemia,** Kalliopi Manola, Greece
- 19:30 Optical Tweezers: A Promising Tool in Cell Biomechanics and Biomedical Research,** Eirini Spyratou, Greece

Friday, 6 December 2013

HALL-B

8:30-11:00 Microbiology/Cytology/Immunology Oral Presentations

Chair: Prof. Loukia Zerva, Dr. Aikaterini Karagiorgou

- 08:30 Development of a Nanoscale Optical Fiber Biosensor Assay for Rapid Detection of Infectious Agents, Thomas Inzana, USA**
- 08:50 Levels of Enzyme Activity of Blood Serum for the Ability to Destroy Peptidoglycan in Patients with Purulent-inflammatory Diseases, Victorya Zemko, Belarus**
- 09:10 Identification of Proteins from Bacteria of Unknown Genome Sequences, Medicharla V. Jagannadham, India**
- 09:30 Laboratory diagnosis of Blood and Tissue Parasitic Infections – Do we still need Microscopy? Mirjana Stupnisek, PhD, MLT, Croatia**
- 09:50 Attitudes, Knowledge and Perceptions of the HPV Vaccine in Greek Adolescent Girls and Their Mothers: A Pilot Study, P. Gioldasi, Greece**
- 10:10 Cytological samples are important for early detection of Malignant Mesothelioma, Kyoko Komatsu, PhD, Japan**
- 10:30 Levels of IgM natural autoantibodies against actin and fetuin detected in sera of patients with cancer, Diogena Prifti, Greece.**

11:00 - 11:30 Coffee break

HALL -B

11:30-13:30 Molecular Biology Oral Presentations

Chair: Prof. Phaedra Eleutheriou, Dr. Evangelos Bozas

- 11:30 Prevalence of ARG72PRO Polymorphism of Tumor Suppressor Gene P53 in a Random sample of General population, Prof. Androniki Papoutsis, Greece**
- 11:50 Polymorphism 4G/5G of Plasminogen Activator Inhibitor-1 (PAI-1) Gene in general population Sample of Northern Greece, Prof. Androniki Papoutsis, Greece**
- 12:10 Distribution of Insertion/Deletion Polymorphism of Angiotensin I converting Enzyme Gene (ACE I/D) in Health Greek Population, Prof. Androniki Papoutsis, Greece**

Molecular Biology lectures

- 12:30 Real time-PCR molecular technique and diagnostic applications, Petros Papalexis, MSc, Greece**
- 12:50 Humans, C. elegans and DNA: a worm's tale, Dr. Thomais Kakouli-Duarte, Ireland**

Saturday, 7 December 2013

Round Tables, Oral & Poster Abstract Presentations and Closing Ceremony

9:00-11:30, Oral abstract presentations of the 4th Panhellenic Medical Laboratory Technologists Conference

Chair: Prof. Christina Foutzoula, Prof. Nikolaos Thalassinos, Prof. Eleni Vagdatli

09:00 Επεξεργασία σκελετικού μυός, Σπύρος Καλαντζάκης

09:15 Επιπλασμός της μετάλλαξης A1298C του γονιδίου της 5,10-μεθυλενο-τετραυδροφυλλικής αναγωγάσης (MTHFR) σε νεαρά άτομα με ιστορικό συγγενούς θρομβοφιλίας, Ανδρονίκη Παπουτσή, Νικολέττα Αντου, Α. Καλιφατίδου, Α. Παντελιός, Γεωργία Κιουμουρτζή, Ανδρέας Πεχλιβάνης, Μαρία Κουτσουνίδα, Μαγδαληνή Παπαγρηγορίου, Ελένη Βαγδατλή.

09:30 Εθνικό πρόγραμμα ελέγχου νεογνών (Ε.Π.Ε.Ν.): επιδημιολογική διερεύνηση ενζυμικών τύπων γαλακτοζαιμίας 2006-2012, Δημήτριος Βασιλάκος, Μαρία Καλογεράκου, Μαρία Γουναροπούλου, Βασιλική Γκιώνη, Κλεοπάτρα Σούλπη

09:45 Η επίδραση της χρήσης χλωρίου στην απολύμανση έτοιμων προς κατανάλωση τροφίμων, Παναγιώτης Πίτσος, Αθηνά Μαυρίδου, Αγγελική Μπίρμπα, Απόστολος Βανταράκης

10:00 Εργασιακά ατυχήματα που καταλήγουν σε λοίμωξη σε νοσοκομεία της Ελλάδας αστικού συγκροτήματος μεσαίας δυναμικότητας, Κωνσταντίνος Δαβράδος, Γεωργία Κιουμουρτζή, Νικολέττα Αντού, Αλέξανδρος Παντελιός, Μαγδαληνή Παπαγρηγορίου

10:15 Κρούσματα ηπατίτιδας C και B κατά τα έτη 1998 – 2011 στην Ελλάδα, Δημήτριος Βασδέκης, Μαρία Χατζηδημητρίου, Πέτρος Παπαλέξης, Παναγιώτα Δημητριάδου, Μαρία Τσιλιγγίρη, Στέλλα Μακρή, Στέλλα Μήτκα

10:40 Διαχείριση αποβλήτων σε τριτοβάθμια υγειονομική μονάδα, Δημήτρης Φούρκας, Δημήτρης Τόγκας

11:00 Βιολογικοί κίνδυνοι και επίπεδο ανοσοπροφύλαξης των εργαζομένων σε οδοντοτεχνικά εργαστήρια. Α. Κουτσοπόδης, Β. Δρακόπουλος, Α. Καλογέρης, Κ. Χεριδης, Π. Παπαλέξης, Χ. Σκανδάλη, Π. Δρόσος, Θ. Κωνσταντινίδης

11:30-12:30 Coffee break

12:30-14:00, Cytology Round Table

The role of cytotechnologist in the modern cytopathology laboratory

Chair: Prof. Petros Karakitsos

12:30 Role of technologists in flow cytometric analysis

Dr. Aris Spathis, University General Hospital "ATTIKON", Greece

13:00 Molecular biology techniques in a modern Cytology Lab

Dr. Christine Kottaridi, University General Hospital "ATTIKON", Greece

13:30 The role of technologists in quality assessment and assurance schemes of a Cytopathology Lab

Stella Kazika, University General Hospital "ATTIKON", Greece

Programme

14:00-15:15, Medical Analyzers and Informatics Round Table

The state of the art in Clinical Laboratory and the upcoming technological Trends and Challenges

Chair: Spyros Matsagos, MSc, Greece

14:00 An overview of crucial functional components of modern Clinical Chemistry automated analyzers, Dr. Aris Tzavaras, Greece

14:25 Laboratory Information Management Systems (LIMS) as an essential component of contemporary Health-care, Maria Botsivaly, BSc, MSc, Greece

14:50 Tomorrow's IVD Technology as reflected on relevant Patent-Documents: Concerned about the future? Prof. Basilis Spyropoulos, Greece

9:00-15:00, Laboratory Tour and Museum Visit

15:30 Closing Ceremony

**Regional European Congress of Biomedical
Laboratory Science & the 4th Greek Medical
Laboratory Technologists Conference**

"Technical Advances and current practices"

5-7 December 2013, Athens, Greece

Lectures

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Opening Lecture

Kinetic and genomic approaches to optimize pharmacotherapy. The role of the biomedical laboratory scientist.

George Albert Karikas, PhD.

Department of Medical Laboratories, Faculty of Health and Caring Professions, Technological Educational Institute of Athens, Greece

Pharmacokinetic parameters such as absorption, distribution, metabolism, and excretion (ADME) along with pharmacogenetics-pharmacogenomics are currently playing an important role in the development of personalized medicines and future pharmacotherapy. Personalized medicine uses information about a person's genes, proteins, enzyme activities, and cellular environment to diagnose, and treat disease including targeted cancer monoclonal therapies.

Pharmacogenetics informs development of safer prescribing criteria and more effective drugs, whereas pharmacogenomics reveals the variations in DNA sequence related to drug response. Common genetic variations are single-nucleotide polymorphisms (SNPs), genetic insertions and deletions, and genetic copy-number variations (CNVs).

The success of the personalized therapy is possible through the application of current technology, which can provide a bridge between metabolism status and an individual's response to a particular drug and therapeutic treatment. The incorporation of pharmacogenomics into the drug development process has the potential to improve target identification, accelerate the development process and reduce the attrition rate.

Commercially available technology that is available for in vitro quantification of drug and drug metabolite levels in blood and plasma include: high-performance liquid chromatography, mass spectrometers, flow cytometers, SPR biosensing instrument, ELISA. However, these technologies are not very practical for personalized medicine applications, because they are expensive; require specialized reagents labeled with fluorescent or radioactive tags, semi-quantitative, time-consuming and unable to distinguish between substrates and inhibitors.

The solution to decentralization of hospital-based tests for the evaluation of drug metabolism is through design and development of new portable biosensor technology capable of evaluating pharmacokinetic parameters (ADME), along with measuring the toxicological effect of a drug in a real-time.

Present presentation provides a sort overview on recent kinetic and pharmacogenomic approaches regarding current applications and future prospects towards personalized medicine-pharmacotherapy-an interesting and promising field for the biomedical laboratory scientists.

Vaiopoulou A, Gazouli M, Karikas GA. J BUON. 2013 Jul-Sep;18(3):570-8.

Session (Using animals in Biomedical Research)

New legal framework on the use of animals in Biomedical Research

Chrysa Voyiatzaki

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A new Presidential Decree (PD) 56/2013, in harmonization to the new European Directive 2010/63, now regulates the use of laboratory animals in biomedical research. Main topics, which are promoted and regulated by the new legislation are: the replacement and reduction of animals' use in procedures and the improvement of the breeding, the housing, the care and the use of animals in procedures, the origin, the breeding, the tagging of the animals, the care and housing of the animals and their killing, the operation of breeders, suppliers and users, the evaluation and the authorization of protocols which include the use of animals in procedures. The PD is applicable on cases where animals are used or are intended to be used in procedures or are specifically bred in order to use their organs or tissues for scientific purposes until the animals are killed, given for adoption or return to an appropriate habitat or a livestock system. The liable operators of breeding, supply and use establishments get the operation permit for their installations from the competent regional authority of the Region for a period of five (5) years, granted only under condition that the requirements of the said decree are met in order to ensure the welfare of the animals which are housed and kept in their installations. They have also to be equipped with qualified veterinarian specialized in the medicine of laboratory animals, charged with advisory duties as well as with a committee to follow up and give opinions about the welfare of the animals. Education and training of personnel working with laboratory animals is also of high priority. A National Committee is established, in the Ministry of Rural Development and Food, for the welfare of the animals used for scientific purposes.

Session (Using animals in Biomedical Research)

Education and training guidelines for those involved in animal experimentation

Ismene Dontas, DVM, PhD

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The previous Greek legal framework, in conformance to the Directive 86/609/EEC regarding the use of animals for experimental and other scientific purposes, required that the persons using them were graduates of certain scientific specialties. According to the current Greek legal framework in conformance to the new Directive 2010/63/EU, persons performing any of the functions described in the Directive in regard to the use of animals are required to be educated and trained accordingly before they perform their duties. Additionally, the European Commission has established an Expert Working Group with the purpose of issuing guidelines to assist the Member States with a common framework on the minimum education and training requirements of persons, their supervision, competence assessment and continuing professional development, which will protect animals and also facilitate free movement between countries. Their work to date has produced a detailed document regarding the academic qualifications of the above persons, the modular training system which is recommended – with its three main categories of core, function-specific and additional modules – and description of the learning outcomes that should be achieved by each module. It is intended that this document will harmonize education and training in the EU and will ensure animal welfare.

Session (Using animals in Biomedical Research)

Early recognition and classification of pain

Irene Symeon

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Early recognition and assessment of pain in laboratory animals is essential, not only because it is a moral and ethical obligation to relieve pain in animals, but also in order to limit its effects on research outcomes. Pain in animals varies in character, source, duration and intensity. Different animal species vary widely in their response to pain, while animals of the same species may show different responses to different pain stimulus. Although there are no generally accepted objective criteria for assessing the degree of pain that an animal is experiencing, behavior and clinical observation as well as measurement of physiological parameters can be considered as fairly reliable ways that could be used. Tests that measure momentary nociceptive responses are also available for rodents, and even though they have limited application for evaluating pain in most situations, they can provide insight into potential pain-related behaviors. Recently, two facial-expression-based pain coding systems were developed in order to recognize and assess pain in rats and mice. The Rat and Mice Grimace Scales are shown to be reliable, accurate and sensitive methods, while it is believed that facial expression coding of pain can be applied in other animal species too. The ability to assess pain in laboratory animals will probably be improved with the development of validated, objective schemes for particular species and types of procedures. Finally, pain assessment requires detailed knowledge of many different species- specific behaviors. The collaboration of different experts including behaviorists, veterinarians and animal care staff is more than necessary.

Session (Using animals in Biomedical Research)

Ethics in animal based research

Nikolaos Kostomitsopoulos

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Despite all the benefits, the use of animals in biomedical research is still a subject of debate with respect to its true value. The sensitivity of the community and the interest of scientists who work in the field of laboratory animal science and welfare have clearly demonstrated that the use of animals in biomedical research must be conducted under specific scientific, legal and ethical rules. The ethical justification of a research project starts from its initial designing phase until its completion and the review of the obtained results. Justification of the necessity of the project and the need to use animals for the human or animal health, the importance of conducting a pilot study and a systematic review of previously published animal research on the topic, and the availability of the proper facilities, equipment and personnel are the main issues of concern in the ethical review of a research project. The ethical justification of the proposed project by the scientists themselves involves teamwork, and should be a sustainable rather than being a one-off procedure. This justification reflects the interest and the responsibility of scientists to reduce the number of animals, refine the procedures, and possibly replace animals in their research projects. The end results of the ethical review process will be the creation of a trust relationship between scientists and society.

Session (Experimental animal models for designing human vaccines: from Edward Jenner to tomorrow's vaccines)

Advances in antigen selection for developing effective vaccines

Maria Agallou, PhD

Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, Greece

Vaccination has been one of the most effective interventions to decrease mortality and morbidity due to infectious diseases in the history of mankind. In any vaccine, the selection of antigens is a crucial step. In the past, although a rational approach was used, vaccine antigens were identified largely with empirical approaches. However, these methods were limited by the fact that some pathogens did not have easily identifiable immunogenic or protective vaccine antigens. Reverse genetics and reverse vaccinology are now used to generate rapidly new vaccine strains and to mine whole genomes in the search for promising antigen. Additionally, the application of structural vaccinology could boost the development of vaccines against diseases in which other approaches have not been successful. Also, the development of next-generation sequencing and proteomic techniques has enable researchers to mine entire microbial genomes, transcriptomes and proteomes to identify novel candidate immunogens. Vaccine design has therefore become more tailored, and in turn has opened up the potential of extending its application in immunotherapies to tackle diseases such as cancer, Alzheimer disease and immune-mediated disorders.

Session (Experimental animal models for designing human vaccines: from Edward Jenner to tomorrow's vaccines)

Vaccine adjuvants as tools for designing innovative vaccines

Koutsoni Olga

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The goal of vaccines is to generate a strong immune response providing long-term protection against infection. Weakly immunogenic antigens can be overcome by the use of adjuvants. Adjuvants are components included in the vaccine formulation to strengthen the immune response directed to an antigen. The biological properties of the adjuvants can be explained by three mechanisms: a) provision of a depot of antigen at the site of inoculation creating an antigenic reservoir for slow release, b) facilitation of antigen targeting, processing and presentation to antigen presenting cells (APCs) and c) creation of a microenvironment conducive to modulation of the elicited immune response. Adjuvants can be classified according to their component sources, physiochemical properties or mechanisms of action. The adjuvants that are used in modern vaccines are classified in two different types: immunostimulants (e.g. Toll-like receptor ligands, saponins, bacterial toxins) and vehicles (e.g. aluminium salt based compounds-alum, liposomes, virosomes, oil-in-water emulsions). During the last eighty years only a few adjuvants have been accepted for use in human licensed vaccines (e.g. alum, MF59, MPLA). There is no unique set of characteristics that would describe an ideal vaccine adjuvant. Recent developments in both synthetic and naturally derived adjuvants, polymers and polyesters (microspheres) suggest that single dose vaccines may be realized. T

Session (Experimental animal models for designing human vaccines: from Edward Jenner to tomorrow's vaccines)

Novel particulate vaccines utilizing nanotechnology

Maritsa Margaroni

Laboratory of Cellular Immunology, Department of Microbiology, Hellenic Pasteur Institute, Greece

The impact of vaccines in global health is beyond question. Since Jenner in 1796 to the present, vaccines have permitted the control of many infectious diseases and even the eradication of others. While important achievements in vaccination have relied on the use of attenuated vaccine or whole killed pathogens, nowadays research is mostly focused on subunit vaccines, which are purer, safer and easier to produce than classical vaccines. Unfortunately high purity often renders them poorly immunogenic, and thus dependent on adjuvants. The most commonly used adjuvants are aluminium based, but these may cause local reaction or not strong enough immune response. Recently attention has been drawn to the utility of nanoparticles (NPs) for vaccines. Nanoparticles have several advantages over conventional antigen delivery carriers. Antigen is protected against degradation in vitro and in vivo, while controlled antigen release can be achieved. Moreover, antigen encapsulated or decorated to NPs are more efficiently uptaken by APCs and certain modifications to NPs surface may facilitate targeting of specific immune cells. There are several types of nanoparticles that have been studied along with antigens related to infectious or other diseases (cancer, diabetes) of human or veterinary interest. Results have been promising and some formulations have undergone clinical trials. The increased number of publications regarding nanoparticles raises hopes for discovering novel, more efficient vaccines. The generation of new subunit vaccines and the implementation of new delivery routes still make the development of safe and efficient adjuvants for vaccine design, a priority.

Session (Biomedical Scientist in Europe)

Use of Ethical Reflection Model to Develop Ethical Competence

Marie Nora Roald

The Norwegian Institute of Biomedical Science, Norway

Ethical reflection models can be used as a systematic approach to ethical dilemmas. The method can be used to discuss own and others' dilemmas, reflect on choices and values, and develop communication skills and wisdom.

To help make ethical reflection accessible for Norwegian Biomedical Laboratory Scientists we have developed a programme for this professional group. In this we combine theory and practical exercises in ethical reflection. We have prepared ethical dilemmas for use in group discussions, and use an adapted ethical reflection model to discuss the dilemmas. The elements in the model include exploration of the situation and action options, clarifying who is involved in the dilemma, disclosure of values and principles involved, determining the consequences, and then selecting action.

A reflection model is meant to assist in the process of uncovering what a dilemma or problem consists of, evaluate alternatives and arrive at a possible solution. Participants in our ethical reflection groups have reported that they found it useful and stimulating, as well as challenging, to discuss relevant ethical dilemma with a systematic approach.

Discussing ethical dilemmas using ethical reflection models gives Biomedical Laboratory Scientists the opportunity to discuss professional ethical dilemmas in a systematic, structured manner, and can contribute in strengthening communications competence.

Session (Biomedical Scientist in Europe)

EDUCATION OF BIOMEDICAL LABORATORY TECHNOLOGISTS IN CROATIA

Mirjana Stupnisek, PhD, MLT

IFBLS Board of Directors; President of Croatian Laboratory Association; Senior Assistant on Department of Pharmacology, Faculty of Medicine of the University J. J. Strossmayer in Osijek, Croatia

Development of biomedical laboratory technologies and diagnostics is an integral segment of modern medicine. Biomedical laboratory technology (BMLT) belongs to the healthcare scientific field related to the work of clinical laboratories pertaining to diagnostics, treatment, monitoring and prevention of diseases. It is an interdisciplinary area comprising medical, natural and technical science, and enable the understanding of normal and pathological function of human organism. It is the basis for the simplest and the most complex diagnoses. It requires permanent professional and scientific development in order to be able to apply the new knowledge and technology, and fosters ethical and moral principles of collegial relations among the health professionals, which are indispensable in cooperation between the users, colleagues and other healthcare professions.

In 2009, Croatian Laboratory Association (CLA) and Faculty of Medicine University J.J. Strossmayer of Osijek (MEFOS) signed a cooperation agreement. According to this agreement, the CLA actively and equally participate in the development of curricula for the university undergraduate and graduate study of BMLT. The curriculum is based on international standards and is comparable to the programs of university studies performed on related educational institutions in Europe and world. The university study of BMLT at the MEFOS is conducted in two cycles: undergraduate (bachelor's degree) study lasting for 3 years (180 ECTS), and graduate (master's degree) study lasting for 2 years (120 ECTS). The aim of the study is to produce a sufficient number of experts who will be competent to independently perform highly complex diagnostic procedures, manage biomedical laboratories, participate in research activities, continue their advancement toward the doctoral studies, following the example of other countries in Europe and worldwide, or be competent to take part in the educational process. With its scientific approach and conducting of clinical training, study in BMLT represents a step forward in university education in Croatia.

Session (Biomedical Scientist in Europe)

Distinctive Professional Competencies of Biomedical Laboratory Technologist In Italy

Alba Marzo², Fernando Capuano³, Gianluca Signoretti⁴, Tiziano Zanin¹, Ambra Amerini⁵

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The Italian Biomedical Laboratory Technologist (TSLB) practice and skills were defined by:

D.M. 745/94 (1994) Most important institutionnel document;

Code and Ethics for the profession;

University Bachelor Course regolamentation

Technology, sophisticated techniques as well as different laboratory organization require different number of TSLB, new distinctive professional competencies and higher level of responsibility.

The aim of the work was to establish a photography of the situation in order to give evidence of the different knowledge and skill framework for TSLB.

TSLB enrolled in the study came from different topics and different part of the country.

The topics involved are: hematology, toxicology, microbiology, anatomical pathology, blood bank, pharmacy and pharmacology, biochemical chemistry, genetics.

A defined work group collected the whole documentation present in the country and observed of the real contribution of TSLB in the analytical process since the collection of the sample till the results realize in all laboratory enrolled.

A final map was discussed and approved during Confederation (ANTeL- ASSIATEL-AITIC) Meeting in 2012.

Since 1994 skills and competencies changed and the simply skills and ability transformed in professional practice, ethics and relationship, as well as decisional moment are present all the phases of the analytical process especially in the quality management of the analytical process.

Up today the activity and responsibility of TSLB begins after the phlebotomist, and ends with the technical validation of analytical results in all Biomedical Laboratory.

New professional standard should be improved in the future through the Continuous Professional Development.

Session (Recent data on Blood Safety. Storage and viruses)

Red blood cell storage: the Integrated Omics perspective

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Background: Erythrocyte concentrates for transfusion purposes represent a life-saving therapeutics of primary relevance in the clinical setting. However, efforts have been continuously proposed to improve safety and efficacy of long-term stored red blood cells. In order to improve the quality, safety and efficacy of long-stored erythrocyte concentrates, two main intervention scenarios have been described: (i) oxygen removal in order to pursue anaerobic storage and/or (ii) the formulation of alternative additive/rejuvenation solutions, such as those implying the supplementation of antioxidants (including vitamin C and N-acetyl cysteine).

Metabolomics analyses of erythrocyte concentrates (especially through NMR [1]) have always represented a key to the understanding of the molecular events promoting erythrocyte apoptosis during cold liquid storage.

Design and Method: By means of liquid chromatography coupled with high resolution Q-TOF mass spectrometry [2-6], we were able to highlight metabolic fluctuations of a subset of low molecular biochemicals, including sugars, lipids, nucleotides, aminoacids, etc. both in red blood cells and supernatants, by assaying variations over storage duration against day 0 controls on a weekly basis. After determining the main metabolic changes throughout red blood cell storage duration, we thus performed similar analyses on deoxygenated erythrocyte concentrates stored in the presence of helium [6,7], and on erythrocyte concentrates stored in CPD-SAGM solution supplemented with ascorbic acid and NAC [8].

Results: In erythrocyte concentrates stored under classic conditions (CPD-SAGM at $\approx 4^{\circ}\text{C}$ for up to 42 days), we could confirm and expand existing literature about the rapid fall of glycolytic rate and accumulation of glycolysis end products [1,2]. A shift was observed towards the oxidative phase of pentose phosphate pathway, in response to an exacerbation of oxidative stress (altered glutathione homeostasis and accumulation of peroxidation/inflammatory products in the supernatant). However, this phenomenon was not sufficient to successfully cope with oxidative stress, which was indeed highlighted by the accumulation of reactive oxygen species, and their relative protein targets (as gleaned via the determination of carbonylated proteins, haemoglobin glycation, protein fragments) or oxidized lipid (malondialdehyde, oxidized eicosanoid derivatives). This was reflected in 2DE proteomics profiles, morphological alterations toward the acquisition of spherocytic and spherocytotic phenotypes (as assessed via Scanning Electron Microscopy) and utterly affected RBC capacity to cope with osmotic stress (increased osmotic fragility).

Anaerobic storage of red blood cells through deoxygenation of erythrocyte concentrate units has been demonstrated to potentially extend the shelf life of erythrocyte concentrates up to 63 days [9], by better preserving ATP and DPG reservoirs [9]. We could confirm previous evidences about long term anaerobiosis promoting glycolytic metabolism in RBCs and prolonging the conservation of high energy phosphate reservoirs and purine homeostasis [6].

However, almost counter-intuitively, oxygen removal does not result in lower levels of oxidative stress, especially upon reoxygenation of the units [6,10]. Indeed, hypoxia limited antioxidant capacity of red blood cells as it blocked the metabolic shift towards the pentose phosphate pathway (PPP) [6,10], which is responsible for the production of the reducing coenzyme NADPH, that in turn is essential to maintain the homeostasis of several anti-oxidant enzymes and pathways (glutathione homeostasis, for example).

In vitamin C and NAC supplemented erythrocyte concentrates, we could observe decreased energy metabolism fluxes (glycolysis and PPP), depressed by the competitive uptake of ascorbate and glucose [8]. Supplementation of anti-oxidants was effective in modulating the redox poise, through the promotion of glutathione homeostasis, which resulted in decreased hemolysis, lower accumulation of malondialdehyde and oxidation byproduct metabolites (including GSSG and prostaglandins). Erythrocyte morphology was

better preserved during the first four weeks of storage, though no significant improvements were observed at the end of the storage period, as gleaned through scanning electron microscopy [8].

Conclusions: The present body of preliminary investigations will pave the way for future studies aiming to assess the validity of newly proposed additive solutions or alternative storage strategies through monitoring of metabolism through untargeted metabolomics. Preliminary results suggest that antioxidants improve storage quality by coping with oxidative stress at the expenses of energy metabolism. On the other hand, oxygen removal might preserve red blood cell morphology better than the supplementation of anti-oxidants, since ATP preservation represents one key avenue to prevent cytoskeletal changes in red blood cells over storage duration. On the other hand, although oxidative stress represents a critical challenge for RBCs under blood bank storage conditions, boosting redox metabolism instead of energy metabolism does not seem to be sufficient to prevent and cope with those lesions targeting membrane morphology.

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Session (Recent data on Blood Safety. Storage and viruses)

Emerging viral infections: A potential threat for blood safety

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Highly sensitive molecular and serological detection methods in blood donor screening, have minimize the infectious risk of several transfusion-transmitted pathogens. During the last two decades the safety of blood products has improved dramatically regarding human immunodeficiency virus (HIV), human T-cell lymphotropic virus (HTLV I,II) and hepatitis B and C viruses.

However, blood borne infectious diseases remain a major subject of interest for blood safety, since new infectious agents have been identified as a continuing threat to recipients of blood products. Currently, attention has turned to emerging infectious diseases (EIDs) whose incidence in humans has increased within the past 25 years or threatens to increase in the near future. Among the newly recognized blood-linked infections is the variant Creutzfeldt-Jakob disease (vCJD), mediated by a prion agent responsible for

transmissible spongiform encephalopathies leading to fatal animal and human neurodegenerative disease. Other emerging viral agents which may cause complications to immunocompromised blood recipients are (arthropod-borne) arboviruses such as West Nile Virus (WNV), Dengue (DENV) and Chikungunya (CHIKV) virus, which have been imported in Europe from tropical and subtropical areas with periodic large outbreaks and their risk varies by season and location. Cytomegalovirus (CMV), a common virus with high prevalence rate in donor population, is connected to significant complications in the immunocompetent patients as well.

In addition, other emerging infectious agents with lower scientific and epidemiologic evidence of risk in regard to blood safety for which there is public or regulatory concern in the future are, HIV new variants (African O and N, M type), HTLV 3 and 4, or simian foamy viruses (SFV). Concerning Hepatitis A (HAV) and hepatitis E (HEV) viruses, although transmission through blood is uncommon, may pose a particular threat to blood products due to their detection in plasma pools and their high resistance to inactivation procedures. Chronic infections with hepatitis E virus have been reported among transplant recipients, immunodeficient individuals and infants in neonatal intensive care units.

Furthermore, transfusion transmission of Human parvovirus B19 from blood components can cause serious complications among specific patient groups, like those with hemophilia, sickle cell disease or thalassemia, bone marrow transplant recipients and other immunocompromised individuals. The relatively high prevalence of B19V infection in the general population and eventually in blood donors has raised the need of plasma qualification testing for B19V DNA, in order to improve the safety of plasma derivatives. Moreover, human herpes virus 8 (HHV-8) infection is associated with Kaposi's sarcoma and Castleman's disease particularly in HIV seropositive individuals.

Concerning Influenza A virus subtype H5N1, although it is a highly pathogenic avian agent that could lead to high community transmission and worldwide influenza pandemic, the risk related to blood transfusion was judged low at the present.

The safety of blood supply has been challenged by identifying and preventing emerging infections among blood donors and blood recipients. In this regard several measures are being taken to reduce infectivity of blood products, including the exclusion of at-risk donors, the use of highly sensitive diagnostic tests, as well as several physical and chemical methods aiming in the inactivation of potential emerging infectious pathogens present in blood without changing significantly its physiological properties.

Session (Recent data on Blood Safety. Storage and viruses)

Recent findings in HIV Cure research towards HIV/AIDS pandemic evolution

Apostolos Beloukas, UK

HIV/AIDS pandemic evolves and recent findings on HIV infected patients treatment as well as on follow up of treated and untreated patient and global prevention policies have dramatically change the spread of epidemic.

HIV incidence, or new infections, have decreased by more than one-third overall and by half among children over the past decade, and widening access to antiretroviral therapy has helped push AIDS-related mortality down by 30% since its peak in 2005, according to a new report released (23 September 2013) by UNAIDS.

New HIV infections among adults and children were estimated at 2.3 million in 2012, a 33% reduction since 2001. New HIV infections among children have been reduced to 260,000 in 2012, a reduction of 52% since 2001. AIDS-related deaths have also dropped by 30% since the peak in 2005 as access to antiretroviral treatment expands.

By the end of 2012, some 9.7 million people in low- and middle-income countries were accessing antiretroviral therapy, an increase of nearly 20% in just one year. In 2011, UN Member States agreed to a 2015 target of reaching 15 million people with HIV treatment. However, as countries scaled up their treatment coverage and as new evidence emerged showing the HIV prevention benefits of antiretroviral therapy, the World Health Organization set new HIV treatment guidelines, expanding the total number of people estimated to be in need of treatment by more than 10 million.

As concerns HIV/AIDS testing, on 21 August this year, the U.S. Food and Drug Administration (FDA) approved the 1st rapid diagnostic test that detects antibodies against both HIV-1 and HIV-2, as well as the HIV-1 p24 antigen. Detection of the antigen but not antibodies indicates acute infection, allowing for the possibility of improved prevention and earlier treatment. Moreover, it has been proposed by the American Society of Paediatrics that adolescents and young adults should be offered risk reduction counselling and routine testing in an effort to prevent HIV transmission and to initiate treatment in a timely manner. Furthermore, earlier this year the U.S. Preventive Services Task Force (USPSTF) issued a highest-level, recommendation that all adolescents and adults ages 15 through 65 years should receive routine HIV screening.

Last 30 years, there have been many scientific discoveries to slow and possibly curb the spread of the epidemic. However while some approaches have been highly successful, vaccines have not offered so much hope or good news. Last years researchers seem to be focused rather on the development of therapeutic vaccine instead of a prophylactic one. Apart from this, different routes have been recently proposed and followed in order to prevent or limit HIV spread. Prevention through the daily use of antiretrovirals in HIV-uninfected people, belonging to high risk groups, to block the acquisition of HIV infection; this prevention approach is known as pre-exposure prophylaxis (PrEP). At this stage evidence is available from studies with two groups: men and transgender women who have sex with men; and serodiscordant heterosexual couples. Furthermore, Post-Exposure Prophylaxis (PEP), which means taking anti-HIV medications as soon as possible after you may have been exposed to HIV to try to reduce the chance of becoming HIV positive.

As concerns totally HIV cure gene therapy and bone marrow transplantation consist the two main areas, where researches are focused at the moment. "The Berlin Patient," is thought to be the 1st individual functionally cured of both HIV and leukaemia after receiving a stem-cell transplant from a donor who is genetically resistant to HIV. Additionally, earlier this year two additional H.I.V.-infected patients in Boston ("Boston patients") who had bone-marrow transplants for blood cancers have apparently been virus-free for weeks since their antiretroviral drugs were stopped. Driven by these unique cases Sangamo BioSciences has developed a technique that uses a zinc finger nuclease (ZFN) to cut out the gene in CD4 cells that controls expression of the CCR5 co-receptor, one of the gateways HIV uses to enter cells, leading to a new road of genetic modification of CD4 cells in order to control HIV infection and as consequence control HIV/AIDS pandemic.

Session (Doping control. Procedures for a valid and useful result)

Doping control Test Distribution Planning

Dr. Efstathios Koukeas, Analyst in the General Chemical State Laboratory

Test Distribution Planning in Doping Control is performed by Anti-Doping Organizations according to the mandatory World Anti-Doping Code and International Standard for Testing provisions. The objective is to plan and implement an effective distribution of sample collections between In-Competition and Out-of-Competition, Urine and Blood, Target and Random in each nation, sport, or discipline within the sport, resulting in the effective detection, deterrence and prevention of doping. The main activities of such planning are information-gathering, monitoring and follow up, risk evaluation; and developing, monitoring, evaluating, modifying and updating the Test Distribution Plan. Indispensable to the Test Distribution Planning is the development, according to published criteria, of the Registered Testing Pool, namely the pool of elite Athletes, who are required to provide a quarterly Whereabouts Filing including the addresses of the places where they will be residing, training, competing or performing other regular activities along with the relative time frames and also one specific 60-minute time slot between 6 a.m. and 11 p.m. each day where they will be available and accessible for Testing at a specific location.

Session (Doping control. Procedures for a valid and useful result)

The sample collection process in Doping Controls

Nikolaos Bournousouzis., DCO (Doping Control Officer), Medical Laboratory Technologist (Sismanoglio General Hospital) MSc Pathobiochemistry.

The global fight against doping in sport is the important centerpiece of NADOs (National Anti-Doping Control Organizations) part of which is the anti-doping efforts via sample collection, also known as doping control. This explains the sample collection process that athletes chosen for doping control will undergo, including both urine and blood collection and testing.

The sample collection process is designed to be a safe process and as comfortable as possible for athletes, while ensuring that maximum sample integrity is maintained throughout. The application of the International Standard for Testing (IST) of WADA is mandatory for the Greek antidoping-organization, ensuring the effectivity of testing as well as maintaining the integrity and identity of doping control samples.

My goal is to analyse the sample collection process as a standard procedure from notification of the athlete to the shipment of the sample to the laboratory and to notify the constant observation, crucial to prevent any attempt by the athlete of altering or tampering with any part of the control process.

As it is scientifically proven that it may take only a few minutes to manipulate the sample or mask, the presence of a prohibited substance or the use of a prohibited method, or to alter with the testing process, the sample collection process remains an essential part of the “healthy” athleticism.

Session (Doping control. Procedures for a valid and useful result)

Recent Advances in Doping Analysis

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Doping is the attempt to enhance performance in sport by illegal administration of pharmaceuticals or application of prohibited methods (e.g. blood transfusions). Over the last decades, we have seen the development of doping from the abuse of classical compounds (highly potent stimulants in the beginning, anabolic steroids later) to new classes of prohibited substances like designer steroids, erythropoietin (EPO), growth hormone (hGH) and insulin growth factor (IGF-1) in the 1990s and recently selective androgen receptor modulators (SARMs) and drugs that are still under clinical trials. Doping control faces a major challenge: old compounds and methods are still state of the art and their control needs to be maintained while new analytical procedures must be permanently included. The anti-doping system trying to meet the challenges enriches its arsenal with new “weapons”. The increasing number of out-of-competition tests relative to the in-competition tests, the shift to more blood tests, the introduction of biological passport (endocrine and hematological profile) to even more sports, the cooperation with pharmaceutical industries, the use of non-laboratory data, like the witnesses from co-athletes, to chase cheating athletes, are among the new tools that are used by the anti-doping community in order to become more effective in the fight against doping in sports. An overview of these tools from the analytical point of view will be presented.

Session (Doping control. Procedures for a valid and useful result)

Doping control Results Management

Dr Efstathios Koukeas

Analyst in the General Chemical State Laboratory

World Anti-Doping Code provisions require that Anti-Doping Organizations (ADOs) establish a process for the pre-hearing administration of potential anti-doping rule violations. In the case of an A Sample Adverse Analytical Finding, the ADO shall conduct a review to determine whether an applicable therapeutic use exemption has been granted or there is any apparent departure from the International Standard for Testing or International Standard for Laboratories that caused the Adverse Analytical Finding. If not, the ADO shall promptly notify the Athlete and other relevant ADOs of the Adverse Analytical Finding/anti-doping rule violated and his rights regarding the analysis of the B Sample. A similar review is conducted in the case of an A Sample Atypical Finding, at the end of which the ADO conducts a further required investigation in order to decide whether or not the Atypical Finding will be brought forward as an Adverse Analytical Finding. In the case of other possible anti-doping rule violations, the ADO shall conduct a follow-up investigation at the end of which, if satisfied that a violation has occurred, shall notify all implicated parts in a way similar to the one previously described.

Session (Applied Microscopy Techniques)

Biopsy of peripheral blood smears with advanced microscopes: focusing on the anemia observed in hemodialysis patients

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Introduction-Background: In the recent decades, advanced imaging techniques such as Scanning Electron Microscopy (SEM) and Atomic Force Microscopy (AFM) are employed in Health Sciences for the investigation of biological specimens since they can reveal valuable information not only on the *overall morphology* of cells, as accessed at the *micrometer* level by conventional optical microscopy, but also on the *membrane characteristics* of cells down to the *nanometer* level. However, the every day use of SEM and AFM in clinical practice is not yet established.

Objective: In this work we focus on our current efforts referring to the utilization of SEM and AFM in the biopsy of peripheral blood smears, specifically focusing on the anemia observed in end-stage renal disease patients on Hemodialysis (HD). In HDp chronic anaemia is observed, that most commonly relates to the impaired production of erythropoietin, iron deficiency and decreased lifespan of Red Blood Cells (RBCs).

Material and Methods: SEM and AFM were employed to thoroughly survey intact RBCs (iRBCs) of HDp (N=7), in comparison to healthy donors (N=7) to obtain information on their structural status since it can relate to chronic anaemia.

Results: The iRBCs membrane of HDp is overpopulated with extended circular defects, called 'orifices' that have typical dimension ranging within 0.2 and 1.0 μm . The population of membrane defects as represented by the 'orifices index', R_{or} exhibits a statistically significant relative increase of order $54 \pm 12\%$ for the HDp when compared to the healthy donors. Interestingly, the 'orifices index', R_{or} correlates with the basic indices of the uremic milieu; urea, calcium and phosphorus, but not with creatinine.

Conclusion: These results suggest that the uremic milieu may downgrade the structure of the iRBCs membrane, possibly triggering biochemical processes that result in their premature elimination from the circulation, thus worsening anaemia.

Session (Applied Microscopy Techniques)

Evaluation of Protein and Cell Arrays Created on Micro- or Nanostructured Substrates by Fluorescence Microscopy

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Fluorescence microscopy is widely employed to detect proteins and/or cells immobilized on solid supports. In both cases, the signal from fluorescent species attached to specific recognition biomolecules is used for quantification of surface attached biomolecules and/or cells. In addition, in the case of cells, structural details can be also visualized and quantitated based on fluorescence signals. In the context of this presentation, the operation principle of an epifluorescence microscope along with a short review on the properties of the most widely used fluorescent labels in protein and cell arrays will be provided. Moreover, the application of these methods for the evaluation of solid supports patterned in the micro- or the nanoscale for creation of protein arrays or as substrates for guided and/or selective cells adhesion will be presented. The studied substrates are prepared either through photolithographic techniques and/or by plasma treatment and poses unique properties with respect to interactions with biomolecules and/or cells. Depending on the material and patterning method, the surfaces could promote or neglect biomolecule or cell attachment. Thus we can create

protein arrays with very controlled spot size or arrays of cells with specific shapes. In the latter case, results demonstrating the preservation of specific cell characteristics when cultured on these surfaces as opposed to standard culture dishes will be presented. In addition, results from plasma nanotextured surfaces exhibiting high surface area and therefore, increased protein immobilization, higher spot signal and detection sensitivity compared to flat surfaces will be provided. The same surfaces when used as cell culture substrates can lead to selective growth and enrichment of specific cells from mixtures opening the route to isolation of rare cancer cells from complex media for diagnostic or personalized therapy applications.

Session (Applied Microscopy Techniques)

Cytogenetics in patients with *de novo* and secondary acute myeloid leukemia

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Introduction: Acute myeloid leukemia (AML) is a heterogeneous disease with regard to clinical features and genetic alterations. Currently, cytogenetic aberrations constitute one of the most important prognostic factors in AML.

Objective: We performed a cytogenetic study in 619 adult AML patients of whom 503 had *de novo* AML and 116 had secondary AML (*s*-AML) in order to define the chromosomal abnormalities and their frequencies in *de novo* and *s*-AML.

Materials and Methods: Chromosome studies were performed on unstimulated bone marrow cells. Molecular cytogenetic analysis (FISH) was performed for the confirmation of karyotypic results only when it was necessary.

Results & Discussion: The sex ratio (males/females) was 1.2/1. The median age of *de novo* AML and *s*-AML was 59.46 and 68.9 years respectively. The most common FAB subtype was M4 in 23.5% of patients followed by M2 in 22.6%, M3 in 19.7% and M5 in 15.3%. A successful karyotypic result was achieved in 98.2% of patients. Among them, normal karyotypes were found in 183 (30.1%) patients; 165 with *de novo* AML (33.5%) and 18 with *s*-AML (15.6%). Complex karyotypes had 32.6% of *de novo* and 34.5% of *s*-AML patients, while monosomal karyotypes were observed in 19.9% and 27.8% respectively. The most common chromosome aberrations in *de novo* AML patients were +8 (25.5%), -7/del(7q) (15.7%), -5/del(5q) (11.2%), abnormalities of 11q23 (6.5%), inv(16) (5.9%), t(15;17) (5.9%), t(8;21) (5.7%) and +21 (3.3%). In *s*-AML the most common abnormalities were -7/del(7q) (28.7%), -5/del(5q) (28.7%), +8 (27.4%), t(9;22) (6.1%), +21 (6.1%), -Y (4.3%) and abnormalities of 11q23 (3.5%).

Conclusions: *De novo* AML and *s*-AML were characterized by the same chromosomal abnormalities. Secondary AML occurred more frequently in older adults and showed a higher frequency of abnormal karyotypes, mainly with an adverse prognosis, including complex and monosomal karyotypes as well as the poor prognosis aberrations -7/del(7q) and -5/del(5q).

Session (Applied Microscopy Techniques)

Optical Tweezers: A Promising Tool in Cell Biomechanics and Biomedical Research

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Introduction: In the field of biomechanics, the mechanical properties of cells have been implicated in many aspects of human physiology and pathogenic diseases. In biomedicine research area, new drug delivery systems (DDS) have been developed for more targeted and effective therapy. The mechanical and rheological properties of DDS proved to be an important predictor of circulation efficiency through capillaries and drug releasing.

Background: New biophotonic techniques, as optical tweezers, are of great importance for biomechanical measurements in both cells and DDS. Optical tweezers (OT) are non-invasive tools which are developed using focused laser beam for manipulation of matter in micro- and nano-

cosmos. In today's biophysical world, OT become a multifunctional tool used to speed up, slow down, rotate, trap and deform cells and macromolecules.

Objective: In this work, we used OT as a tool to handle cells (erythrocytes) and DDS (liposomes), to induce stretching, deformation, rotation and to study their properties such as the membrane elasticity, deformability and viscoelasticity.

Material and Methods: Novel OT system was used to manipulate simultaneously more than one cell/liposome and cause reversible deformations. Optical forces exerted by OT on trapped cells/liposomes were measured by dielectrophoresis method.

Results and Discussion: Optical force depends essentially on the cell surface and the cytoplasmic refractive index thus the biochemical modification associated with different states of the cell pathology influences its behaviour under OT. By measuring the optical forces for reversible liposome/cells deformations, shear modulus and bending modulus were measured.

Conclusions: The ability of the selective manipulation of cell/liposomes and examination of their biomechanical properties will bring us closer to understanding the cellular-liposome interactions.

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Session (Heamatology) -1

Informatic Crossmatch in Transfusion Medicine

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The health area is a very sensitive area that involves high costs and high risks, is an area where the error cannot exist because it undermines the health, survival and well being of the population.

Systems and information technology in health care have undergone a huge evolution over the years. The impact of new technologies on health have been found in research, help define diagnoses through computer systems for clinical decision support in treatments and in various acts of diverse specialties and services.

The computerization in health care, among many other things, simplify processes, reduce costs, increase production capacity, improve safety and quality control and increasingly reduce the error. These consequences lead to a strong and steady bet in an area as sensitive as the health sector.

In this study will be analyzed two methods for assessing the compatibility of blood components, the classical method and computer (computer crossmatch). The two methods are analyzed separately and are compared to the level of security and risk to the patient, the price and time. It will also be presented the system requirements.

At the end of this study by the results that are presented will be able to ascertain the most efficient method, which is the method that leads to lower costs without jeopardizing the quality of service, the health and welfare of the patient.

Session (Hematology) -2

Cytotoxic Effects of ionizing Radiation in Cancer Cell Lines of Small Cell Lung Cancer and Large Diffuse B cell Lymphoma – A Therapeutic tool

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Small cell lung cancer and diffuse large B-cell lymphoma are two types of rather aggressive cancer, which by norm surgery is not applicable in most cases. Therefore, a therapeutic approach may include a combination of chemotherapy and radiotherapy, or one of the previous separately. Radiotherapy resorts to the use of corpuscular and high frequency electromagnetic radiation, capable of causing direct and indirect biological damage with the intent of inducing cell death of neoplastic cells. With this study it was intended to study the cytotoxic effects of ionizing radiation, with different doses (0,5-30 Gy) in cellular lines of these pathologies (H69 and FARAGE), performing for such assays of proliferation and cell viability (trypan blue exclusion method, clonogenic assay, cell death evaluation by flow cytometry and morphologic assay) and genotoxicity assay (comet assay). Based on the obtained results, we observed a decrease of proliferation and cellular viability after irradiation with different doses, being the most effective doses 15 and 30 Gy, in both cell lines studied. We verified that with a smaller dose (0,5 Gy) the inhibition of proliferation occurred, followed by a cellular recuperation, such as in the control cells. It was also observed that with the increase of the dose of radiation, DNA damage increased and the rate of viable cell decreased, in agreement with an increase of the number of cell in apoptosis.

Session (Heamatology) -3

Erythrocyte Characteristics of Young Female Blood Donors

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Introduction: It has been suggested that the estrogen Estradiol (E₂) protects the erythrocyte membrane from assaults arising by increased mechanical or oxidative provocations in blood peripheral circulation.

Background: Although this suggestion has not been fully established, there are supportive experimental data showing that the estradiol produced during the menstrual cycle may have an important role in regulating erythrocyte glutathione peroxidase activity, which is a critical factor of the erythrocyte antioxidant defense systems.

Objective: This study focused on the properties of reproductive age women erythrocytes in order to clarify whether or no they are statistically different compared to those of age-matched male controls, with respect to factors probably associated with their storability in blood bank conditions used for transfusion purposes.

Materials and Methods: To that purpose, 50 young female blood volunteers, 18 to 25 years old, weighting >50 Kg and with normal blood pressure, donated 12 mL of blood. Their complete hematological profile as well as RBCs-related indexes (MCV, MCH, MCHC) and parameters like the osmotic fragility and plasma free hemoglobin levels were studied by classic hematological and biochemical approaches and tests. The blood donors' lifestyle was evaluated through the completion of a relevant questionnaire.

Results and Discussion: Most of the variants tested were found within normal range except for blood hemoglobin (<12 g/dL in 46% of the samples) and platelets count (>380x10³/L in 18% of the samples). According to the osmotic fragility test, volunteers' red blood cells were especially resistant to the osmotic lysis, irrespectively of the women's' lifestyle profile concerning traditionally adverse factors like smoking and alcohol consumption.

Conclusion: These results have shown that despite volunteer motivation, about half of the female population of young age cannot be regular blood donors due to low hemoglobin levels. However, the erythrocytes of the eligible blood female donors were characterized by increased resistance to the osmotic hemolysis probably as a result of the advanced estradiol and other estrogen levels. Interestingly, "bad habits" such as smoking and alcohol consumption did not seem to interfere with this suggested beneficial effect of estrogen to erythrocyte properties.

Session (Haematology) -4

Plasma Microparticles: A New Challenge on the Way of Centrifuging Blood in Coagulation Testing?

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Introduction: Coagulation tests, according to CLSI Guideline H21A5, must be performed with platelet poor plasma (PPP) so as platelet membrane phospholipids do not affect the results as well as the tests will show reproducibility. For this reason blood samples should be centrifuged at 2000xg. Nowadays, it is known that in PPP there are microparticles of platelets and of other cells as well, whose phospholipids can affect coagulation tests.

The **objective** of this study is to evaluate any possible difference in the results of the routine coagulation tests performed in PPP and of tests performed in plasma poor of microparticles (MPP).

Material and Methods: Blood samples with Sodium Citrate were collected from 100 people (regardless gender). All samples were centrifuged at 2000xg in order to provide PPP. One part of PPP was centrifuged at 12000xg in order to provide MPP. Prothrombin Time (PT), Partial Thromboplastin Time (APTT), Fibrinogen (Fib) and DDimers were measured in all samples on ACL-Advance coagulation analyzer. After the measurements, people were divided in two groups: Group A: with normal values of DDimers and B: with elevated values of DDimers (activation of coagulation). Statistical analyses were performed with the statistical package SPSS 17:1). Comparison of values of PT, APTT and Fib performed in PPP and MPP for the whole samples as well as for the two groups individually and 2). Correlation of the quantitative changes of PT, APTT and Fib (between PPP and MPP) with the results of D-Dimers.

Results: Only APTT was found significant decreased in PPP (compared with MPP) in all samples ($p < 0,001$) as well as in the samples of the two groups ($p < 0,001$), while there was not found any correlation between APTT differences (between PPP-MPP) and DDimers.

Conclusion: The presence of microparticles in blood causes reduction of APTT regardless of activation or non-activation of coagulation. This fact creates problems for the classic way of centrifugation of blood in coagulation tests.

Session (Microbiology/Cytology/Immunology) -1

Development of a Nanoscale Optical Fiber Biosensor Assay for Rapid Detection of Infectious Agents

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Introduction: Infectious agents that are highly antibiotic-resistant or biothreats require rapid diagnosis at the bedside or in basically-equipped laboratories. Photonic biosensors utilizing optical fibers with long-period gratings (LPG) and self-assembled multilayer (ISAM) films can identify infectious agents through specific binding of surface antigens or DNA.

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA), *Francisella tularensis*, and *Brucella* spp. are highly dangerous, requiring rapid identification.

Objective: Our objective is to develop an LPG-ISAM optical fiber biosensor capable of rapid detection of antigens or DNA specific to MRSA, *F. tularensis*, *Brucella* spp., and other agents without the need for PCR or expensive equipment.

Materials and Methods: The ISAM film was synthesized by repeated immersion of the LPG fiber in positively- or negatively-charged polyelectrolytes. The LPG-ISAM was covalently conjugated with species-specific monoclonal antibodies or DNA probes. Nonreactive sites were blocked and pure cultures or swabs of clinical specimens from infected mice were tested to determine sensitivity and specificity. Binding of antigen or DNA to the fiber reduced light transmission through the fiber. Significance was calculated by Student *t* test.

Results and Discussion: Using a specific antibody or DNA probe to each agent, the sensor could detect 100-800 cells/ml of antigen or DNA (without PCR) from the specific agent based on reduced light transmission through the fiber within 1 hour. However, 10^5 cells of heterologous strains caused little or no light inhibition. The specific agent could also be detected from tissue swabs of infected mice, but not from mice infected with heterologous strains ($p=0.02$). The sensitivity and specificity of each assay was close or equal to 100% when at least 10^3 specific cells were present and the cutoff for a positive result was 7% light transmission inhibition.

Conclusion: Our results show that the LPG-ISAM is a promising culture-free assay for rapid and specific detection of infectious agents.

Session (Microbiology/Cytology/Immunology) -2

Levels of Enzyme Activity of Blood Serum for the Ability to Destroy Peptidoglycan in Patients with Purulent-inflammatory Diseases

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Introduction: Alpha-defensins (DFs) are contained in azurophil granules of neutrophils and possess activity against microorganisms of normal human microflora, e.g. *E.coli*.

Background: Considering that one oligopeptide, which comprises several unique amino acids including D-isomer of alanine is attached to each residue of N-acetyl-muramic acid, peptidoglycan (PG) may be a substrate for determining activity of a number of DFs.

Material and Method: Isolation of PG from cell wall of gram-negative bacteria was carried out by method proposed by V. Lvov, B. Pinegina in our modification. The received PG, labeled with 2% solution of Congo red was used as a substrate. Blood was taken from healthy donors and patients with purulent-inflammatory diseases (PID). Enzymes in blood serum destroyed PG, Congo red became soluble, changed its color from colorless to red with a maximum spectrum of absorption at a wavelength of 495 nm. The result was calculated by using the following formula, obtained after construction of calibration graph for the breeding of Congo red:

$$Y = [-0,001 + 0,026 \times Eop] \times 9,921$$

Y - the desired result (in picokatal)

Eop - optical density of sample minus optical density of control

Results: In patients with PID activity of enzymes destroying PG was significantly higher than in donors. After complement inactivation, enzyme ability significantly reduced.

Conclusions: The technique allows to determine antimicrobial enzyme activity of blood serum for ability to degrade PG, which is possibly may be one of factors of nonspecific resistance, that allows microorganisms to fight against infection.

Enzyme ability to destroy PG was significantly higher in patients with PID, than in donors, which is not connected with activity of complement and can be explained by ejection of a number of DFs from granules neutrophils.

Session (Microbiology/Cytology/Immunology) -3

Identification of Proteins from Bacteria of Unknown Genome Sequences

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Proteomics technology allows identification of large number of proteins using mass spectrometry. The proteins are extracted from the source separated by 1D or 2D gel electrophoresis, followed by digestion into fragments using trypsin. Mass spectra of the individual peptides are obtained and they are further fragmented using Collision Induced Dissociation (CID). The sequence of each of these peptides is determined using an appropriate database wherever it is available. *De novo* sequencing is a preferred method of choice for the determination of peptide sequence when database is not available. Even though several approaches are available, determination of the correct sequence by mass spectrometry is beset with a number of problems viz. incomplete fragmentations, poor spectral quality. Chemical modifications of peptides (viz. succinylation, picolinidation), performed by several laboratories, enhances the b-ion intensities in the CID MS and helps in determining the sequence of the peptides. However, most of these studies are carried out on synthetic peptides and the chemical procedures used in these methods are hardly suitable for the mixture of tryptic peptides. We developed a method to improve the b-ion intensities by acetylating the N-terminal of the peptides. The method is simple, fast and can be applied to large number of peptide mixtures as frequently required in proteomics. Thus using a combination of different methods of ionization and peptide sequence validation ~ 2500 proteins were identified from an Antarctic bacterium *Pseudomonas syringae*, whose genome sequence was not known. These methods also improve the efficiency of *de novo* sequence.

Session (Microbiology/Cytology/Immunology) -4

Laboratory diagnosis of Blood and Tissue Parasitic Infections – Do we still need Microscopy?

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Blood and tissue parasites comprise a large number of protozoa and helminths found in tropical and temperate climates worldwide. Certain parasites cause infections with associated high morbidity and mortality, while others may cause mild or asymptomatic disease.

Expert microscopic examination of Giemsa stained thick and thin peripheral blood films is used for detection and identification of the protozoan blood parasites (*Plasmodium*, *Babesia*, and *Trypanosoma*), and the filarial nematodes, whereas microscopic examination and/or culture of ulcer samples, bone marrow, tissue aspirates, and biopsies are useful in the diagnosis of trypanosomiasis, trichinosis, toxoplasmosis, and leishmaniasis. Serologic assays (detection of antibodies) are available as adjunctive methods for the diagnosis of a number of blood and tissue parasite infections. Unfortunately, none of these assays are sensitive or specific enough to be used on their own to establish the diagnosis (shows significant cross-reactivity). Laboratory methods that detect parasite antigens and/or DNA provide an attractive alternative to traditional morphologic and serologic techniques. A simple rapid immunochromatographic card assay for the detection of *Plasmodium* may find use in emergency departments or out-patient clinics to establish a diagnosis of malaria quickly while awaiting results of blood films. CDC and reference laboratories perform extremely sensitive nucleic acid detection methods for certain blood and tissue parasites, but turnaround time may be prolonged. Molecular assays (NAAT) may be of particular use in patients with very low parasitemias or in specifically identifying organisms that cannot be differentiated microscopically, but NAATs should not be used to monitor response to therapy. Microscopy still remains the “gold standard” of laboratory testing for the diagnosis of most blood and tissue parasitic infections. Although this method requires a minimum amount of resources, well trained and experienced technologists must be available to obtain maximum accuracy and efficiency, to prepare and examine slides and to recognize these unusual organisms.

Session (Microbiology/Cytology/Immunology) -5

Attitudes, Knowledge and Perceptions of the HPV Vaccine in Greek Adolescent Girls and Their Mothers: A Pilot Study.

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Introduction and Background: Recently developed vaccines against human papillomavirus (HPV) have increased the awareness of HPV infection. However, since the public health impact of HPV immunization will be greatest if a high coverage of the target population is achieved, the target must be to maintain and improve HPV immunization coverage. For this purpose it is worldwide believed that HPV-related issues should be incorporated earlier in school education.

Objective: This pilot study sought to determine knowledge of and attitude towards human papillomavirus (HPV) infection, HPV-related diseases and HPV-vaccines among female adolescent girls and their mothers at several high schools in Athens.

Methods: A self-administered anonymous questionnaire covered demographics; knowledge about HPV infection, cervical cancer, and HPV vaccine; the perceived risk for contracting HPV infection and/or for developing cervical cancer, the perceived benefits of a vaccination to prevent cervical cancer, and willingness to receive an HPV vaccine were distributed among adolescent girls aged 12-18 and their mothers (n=285).

Results: The sample included 193 adolescent girls (67.7%) and 92 mothers (32.3%). The vast majority of mothers (88%) were aware about HPV infection, but only a small percentage 11% have heard about HPV vaccination, moreover out of them only 46% supports that vaccination has effective immunization. As concerns adolescent girls, according to their statement 27.7% did not use condom in their first sexual intercourse, and 54.7% did not use in generally. Only 36% of those enrolled in the present study have been HPV immunization.

Conclusion: Level of acceptance of HPV immunization was found to be lower than what has been reported in other similar studies conducted in EU. Majority of the mothers still express concerns about potential HPV vaccination side effects and the impact of HPV immunization. As concerns adolescent girls lack of knowledge was observed even to basic, since found not to be aware about HPV, HPV-related issues and HPV-vaccine.

Session (Microbiology/Cytology/Immunology) -6

Cytological samples are important for early detection of Malignant Mesothelioma

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The Number of the patients with malignant mesothelioma in Japan has been increasing almost about doubled in recent 10 years. Japanese Government has given the compensation to the patients who suffered malignant mesothelioma caused by asbestos. Effusion cytology is very important for early detection of it. However, its cytological evaluation, especially, distinguish from reactive mesothelium or adenocarcinoma is difficult. For this situation, we studied the characteristic morphological findings of malignant mesothelioma in effusion cytology. We reviewed cytology specimens of malignant mesothelioma cases during these 5 years. We experienced 11 cases of malignant mesothelioma in our hospital. We investigated morphological features of malignant mesothelioma cells in Papanicolaou smears.

Results and conclusions are as follows: 1. Mesothelioma cells form papillary or insular structure in its most common glandular and papillary histological subtype. We will find the transition from mesothelial cells to malignant mesothelioma cells, many multinuclear or binuclear cells, multiple mutual inclusion bodies are key to diagnosis. 2. Immunostaining is very useful for confirmation. According to the Japanese guideline, we need to use at least three markers for mesothelial cells (Calretinin, Glute-1, D2-40, Thrombomodulin, WT-1,) , and several other markers for cancers. MMP-2, our original data, were immunohistochemically positive for spindle cells in biphasic phenotype of malignant mesothelioma and stromal cells in invasive front in general. MMP-2 were immunocytochemically positive for the nuclei and cytoplasm of malignant mesothelioma cells, while negative for reactive mesothelium. 3. Although malignant mesothelioma is very rare, we should get hold of its cytological image and always consider its possibility, as well as keeping in touch with clinical departments.

Session (Microbiology/Cytology/Immunology) -7

Levels of IgM natural autoantibodies against actin and fetuin detected in sera of patients with cancer

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Background: Natural Antibodies (NABs) are present in the blood stream of healthy individuals and of higher vertebrate animals without any previous intentional immunization. These are mostly antibodies of the IgM, IgG and IgA isotype and are commonly characterized by polyreactivity, reacting with nucleic acids, proteins and haptens. They are frequently encoded by germ-line genes, bearing few or no mutations. Researchers have shown that polyreactive NABs are present in cancer patients, at significantly higher serum levels than in healthy individuals. Particularly, a major subset of natural IgM antibodies in patients with cancer has been described to specifically bind and destroy tumor cells. Moreover, these antibodies have been previously reported to bind specifically to glycoproteins present on the surface of tumor cells but not on normal ones. Thus, these antibodies can be considered as potential cancer markers and could possibly play a role in diagnosis and therapy. Therefore, this study investigates the possible diagnostic role of such natural IgM antibodies in cancer.

Methods: An enzyme immunoassay (ELISA) was undertaken for the detection of NABs of IgM isotype against fetuin and actin in sera from patients with either lung cancer (n=27) or colon cancer (n=47), in comparison with those from healthy individuals (n=52). Fetuin was chosen because of its presence on the tumor cell surface and used as a glycoprotein of reference. On the other hand, actin is considered to be one of the basic target antigens of NABs and its well-known involvement in cancer made this antigen an appropriate choice for this study. The serum levels of IgM NABs were also examined after treatment with acid pH (2.5? or 3.0?PUT IT HERE!!!) buffer solution as denaturing agent (acid-treatment), in order to release any possible blocked antibodies from immune-complexes and thus reveal any 'masked' activity against the two antigens of the study.

Results: Data analysis (using Microsoft Excel 2003 and SPSS) showed that sera contained IgM natural antibodies recognizing actin better than fetuin. Nevertheless, no statistically significant difference was observed for the two target-antigens, on the serum levels of healthy and patients with both types of cancer. Surprisingly, a markedly difference in the serum levels of NABs was observed after acid treatment. In detail, a significant rise of levels of IgM against fetuin was detected in the sera from the two groups of cancer patients compared to those from the healthy controls, whereas, in the case of actin a significant enhancement of IgM levels was observed only after acid treatment of the sera derived from patients with colon cancer compared to healthy control ones.

Conclusion: In conclusion, the present study demonstrates that the detected levels of IgM NABs against fetuin and actin in the sera of patients with lung and colon cancer cannot be used as a diagnostic tool, at least for these two types of cancer. On the contrary, the comparison of the serum levels of IgM NABs tested before and after acid treatment against fetuin and actin might represent a reliable diagnostic marker for at least these two types of cancer. This combined measurement of IgM NABs levels is likely to acquire a larger diagnostic value and therefore lead to a more valid diagnostic assay. Overall, this study highlights for the first time the potential diagnostic value of IgM NABs in cancer and sets the base for the future development of a more sensitive and specific method to determine levels of NABs in immune diseases as cancer. The next step of this study includes the use of the developed immunoassay to other types of cancer (with distinct subgroups of patients), as well as the use of other autoantigens antigens as targets.

O (Informatics/Education/Clinical chemistry) -1

Informatics Procedure in Laboratory Management: Implementation of an Online Course to Maintain Expertise

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Introduction and Background: The Azienda-Ospedaliero-Universitaria Careggi (AOUC), Florence, Italy is a high specialized clinical setting; the Dipartimento Laboratorio is the referral center for AOUC (8 x 10⁶ samples/year). The test ordering and management, is performed online by the informatics procedure DNWEB (Noemalife, Bologna Italy) High personal turnover and work burden may cause an inappropriate use of such procedure, leading to significant errors and delays.

Objective: To improve pre-analytical phase quality, an AOUC multiprofessional group implemented an online course, tailored on specific educational needs and directed to all DNWEB users (2500).

Material and methods: The course teaches how to manage test orders “from brain to bed”; critical points are stressed and links to further explanations are available. Self assessment tests are present; in case of mistake the correct answer and the appropriate section are retrieved automatically. The student needs to pass a final test.

Results and Discussion: During the first 7 months since publication, approximately 10% of DNWEB users unrolled: 210 successfully completed the course, 25 failed and 3 unrolled twice the course.

Conclusion: Better DNWEB procedure understanding and knowledge are supposed to lead to a substantial decrease in pre analytical errors. Although the number of applicants can't be considered substantial, yet a decrease in number of monitored errors can be observed (i.e lack of clinical information) as well as a general higher level of thoughtfulness (i.e quality of barcode printing) was observed. To maintain a high level of expertise, the course needs to become a permanent tool for Continuous Professional Development.

O (Informatics/Education/Clinical chemistry) -2

Predictors of Success for MLS Students

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Clinical Laboratory Sciences, University of Texas Medical Branch, USA

Why are some of our students more successful than others in the MLS Program? Are there predictors that can help to determine success, especially in completing the MLS program and passing their certification examination? This session will present findings on the correlation between applicant, student, and graduate variables (OGPA, SGPA, prerequisite courses, feeder schools,) to outcome measures including program GPA, clinical rotations, completion rates, certification scores, and employment. As would be expected, overall GPA is strongly correlated with science GPA ($r=0.92$). But the correlation between overall GPA and program GPA or certification scores is less strong ($r=0.42/0.64$ respectively). To find other predictors for success a regression model was developed that included additional predictor variables, i.e., age of student (age), gender, minimum B.S. degree obtained before starting the CLS program and medical laboratory technician degree from a community college before beginning the CLS program. This regression model produced an R square of 0.407 with p-value of 0.003, at alpha of 0.05, indicating statistical significance. Using these models will help educators to develop programs and strategies to ensure the success of their students.

O (Informatics/Education/Clinical chemistry) -3

Comparative Study of Serum CA 19-9 and CEA Levels, Measured by Two Immunometric Assays, (ELISA and elfa), in Different Diagnoses of Malignant Pathologies

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Introduction: The continuous evolution of immunological methods of measuring biochemical parameters in biological fluids presents a challenge for chemists and technicians in chemical and biochemical laboratories. Immunoassays are used to quantify molecules of biological interest based on the specificity and selectivity of antibody reagents generated. Assays are designed to detect molecules that are produced intracellularly, or secreted in response to screening compounds {Burtis & Ashwood 1994}. Different methods of quality control are available and routinely used nowadays. It is important that the methods used for assessment of analytical performance are suitable for the intended purpose and for the specific conditions of our laboratories {Abbot Diagnostic Laboratories, 1990}. CA19-9 is a monoclonal antibody generated against a colon carcinoma cell line and it is the main tumor marker of glycoproteins group, associated with increased concentration in malignant gastrointestinal pathologies {Tietz 1995}. Carcinoembryonic antigen (CEA) is a protein found in many types of cells, but associated with tumors and the developing fetus. The CEA is often positive in malignancies other than colonic. In the cancer of the breast, lung, pancreas, stomach and ovary, CEA may be elevated and it can be used to monitor the progress of disease or response to treatment.

Purpose: The purpose of this study was the comparison of serum Ca 19-9 and CEA levels in normal serum samples (control group), measured at the same time with ELISA (enzyme-linked immunosorbent assay) and ELFA (enzyme-linked fluorescent assay), in order to determine whether these methods can be adapted to each other. This study also highlights the importance of the determination of tumor markers CEA and Ca 19-9 in the differentiation and screening (monitoring) of malignant pathologies.

Materials and methods: The study was conducted in normal serum samples taken from 10 apparently healthy patients, {not previously diagnosed with malignant pathologies by abdominal echography, colonoscopy, RMI and FGS, within the range 48-74 years old (control group)}. A 10 ml venous fasting blood sample was taken from the control group patients. CEA and CA 19-9 were measured at the same time with two immunometric assays: ELISA, using analytical immunoenzymatic kit of HUMAN'S company by analytical reader HUMAN READER HS and ELFA using an automated fluorescence reader, Mini VIDAS®, by BioMerieux kit.

In this study, we also determined the level of CEA and Ca 19-9, ALP, LDH in 15 patients (study group) previously diagnosed with malignant pathologies by the echography, colonoscopy, RMI, FGS and surgery within the range 61-78 years old.

ALP and LDH were measured by kinetic method with BTS 310⁺ photometer using Biochem diagnostics kits. Statistical analysis of the analytical data was done by using the statistical software SPSS for WINDOWS.

Results: ELISA analytical results obtained for Ca 19-9 and CEA tumor markers in 10 healthy patients are reported in Table 1. The analytical results for LDH and ALP enzymes measured by kinetic method with BTS 310⁺ photometer are also reported in Table 1. In this Table interval of normal values defined in our laboratory was close to the normal references values that Biochem diagnostics kits sign in,

Table 1. Analytical results obtained for Ca 19-9, CEA, LDH and ALP in healthy patients blood serum samples

STATISTICAL PARAMETER	CA19-9 (U/L)	CEA (ng/mL)	ALP (U/L)	LDH (U/L)
Number of patients (count)	10	10	10	10
Mean value	9.2	2.27	138.7	230
STD	11.76	1.68	55.7	55.4
$\bar{x} \pm \sigma$	9.2± 11.7	2.27±1.68	138.7±55.7	230±55.4
Minimum value	1	0.4	68	150
Maximum Value	39	4.6	260	314

CEA and Ca 19-9 tumor markers were determined simultaneously with two methods, ELISA and ELFA. The analytical results and statistical data treatment are presented in Table 2 and Table 3, respectively.

Table 2. Comparison of CEA values obtained by two immunoassays methods in healthy patient's blood serum samples.

Patient	CEA (ng/mL) measured by		Patient	CEA (ng/mL) measured by	
	ELISA	ELFA		ELISA	ELFA
1	0.4	0.9	6	4.6	4.0
2	2.8	3.1	7	0.6	1.1
3	0.4	1.0	8	3.5	3.1
4	2.9	2.4	9	4.5	3.6
5	2.5	3.1	10	0.5	0.9

Table 3. Statistical summary of CEA values obtained by ELISA and ELFA

Groups	Count	Sum	Average	Variance
ELISA	10	22.7	2.27	2.840
ELFA	10	23.2	2.32	1.506

ANOVA

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.0125	1	0.0125	0.005752	0.940382	4.413873
Within Groups	39.117	18	2.173167			
Total	39.1295	19				

Table 4. Comparison of Ca 19-9 values obtained by two immunoassay methods in healthy patient blood serum samples.

Patient	Ca 19-9 (U/mL) measured by		Patient	Ca 19-9 (U/mL) measured by	
	ELISA	IMMUNOFLUORESCENCE		ELISA	IMMUNOFLUORESCENCE
1	6	15	6	3	13
2	1	11	7	39	43
3	16	25	8	1	10
4	15	23	9	3	13
5	3	13	10	5	14

Table 5. Statistical summary of Ca 19-9 values obtained by ELISA and ELFA

Groups	Count	Sum	Average	Variance
ELISA	10	92	9.2	138.40
ELFA	10	180	18	101.33

ANOVA				A		
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	387.2	1	387.2	3.230256	0.089088	4.413873
Within Groups	2157.6	18	119.8667			
Total	2544.8	19				

The correlation between values of CEA and Ca 19-9 obtained by two different immunometric methods are presented in Figure 1 and Figure 2, respectively.

Figure 1. Correlation between CEA values obtained by ELISA and ELFA in healthy patient blood serum samples.

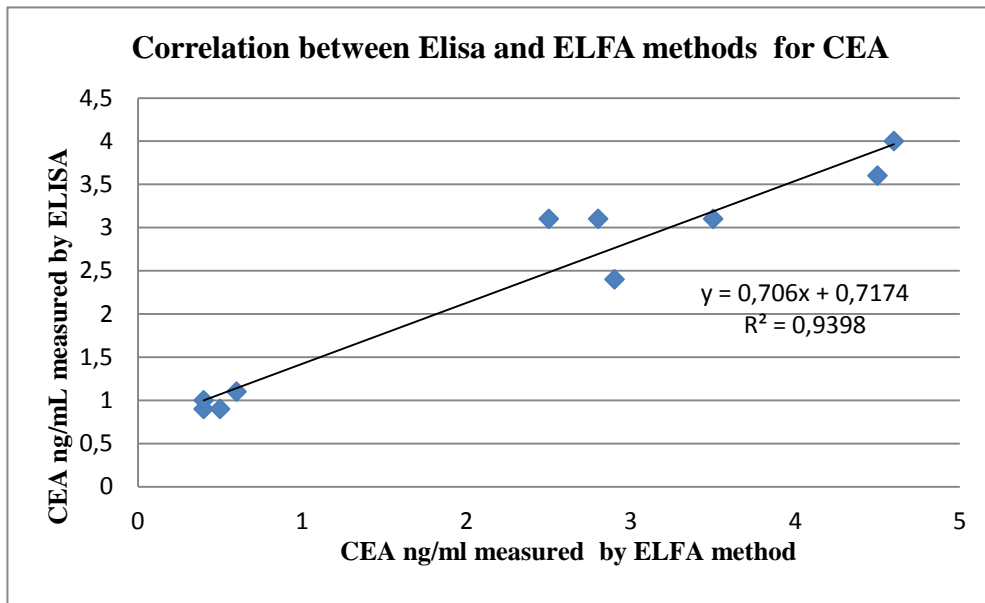
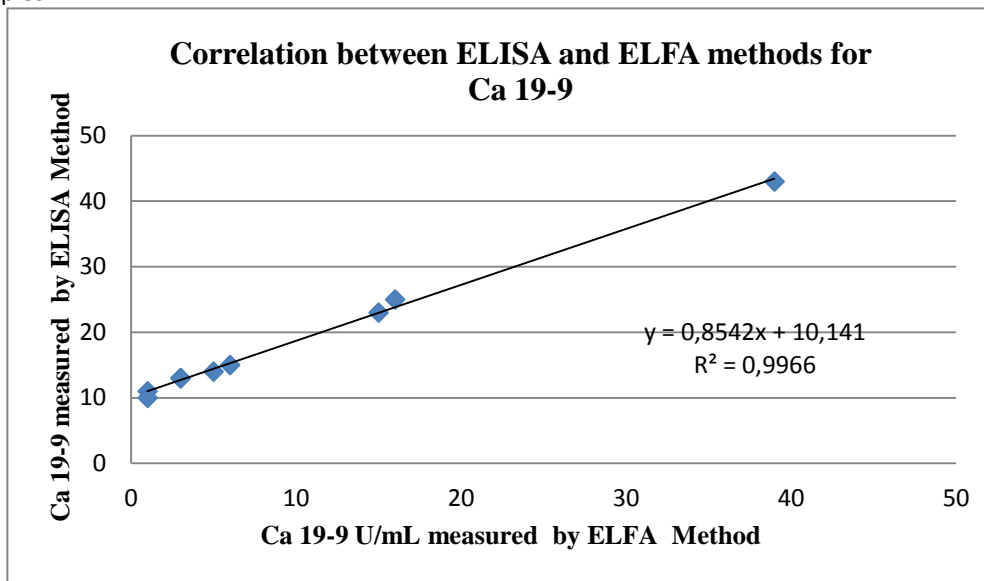


Figure 2. Correlation between Ca 19-9 values obtained by ELISA and ELFA in healthy patient blood serum samples.



There was a statistically positive correlation between two methods. The correlation coefficient was $R^2=0.9398$ for CEA and $R^2=0.9966$ for Ca 19-9.

The second step of this study was the investigation of possible value alterations of tumor markers CEA and Ca 19-9 in malignant pathologies. also we study the possible values alterations for ALP (alkaline phosphatase) and LDH (lactate dehydrogenase) enzyme as adjuvant tumor marker in the diagnosis of malignant abdominal pathologies. For this purpose, a study group (15 patients) was selected, previously diagnosed with malignant pathologies by the echography, colonoscopy, RMI, FGS and surgery within the range 61-78 years old. In their blood serum samples we determined the values of CEA and Ca 19-9, ALP (alkaline phosphatase) and LDH (lactate dehydrogenase).

The analytical and statistical data are presented in Table 6 and Table 7, respectively.

Table 6. Values of Ca 19-9, CEA, ALP and LDH in patients previously diagnosed with malignant pathologies

Patient	Ca 19-9	CEA	ALP	LDH
1	86	15	270	480
2	69	20	310	450
3	75	10	280	470
4	620	207	410	660
5	119	15	320	500
6	40	8	260	900
7	17	5	230	470
8	65	14	270	510
9	5	2	140	530
10	6	3	200	520
11	43	16	260	480
12	14	2	150	510
13	250	50	600	600
14	66	7.2	260	520
15	150	66	300	480

*Normal range respectively are CEA (0.0-5.0 ng/mL) non smoker , Ca 19-9 (0.0-37 U/L)
ALP (100-290 U/L) and LDH(200-400 U/L)

The data in Table 6 confirms that gastrointestinal malignant pathologies tumor marker, CA 19-9, CEA and alkaline phosphatase enzymes have significantly changed in comparison with respective values in the normal patients. Ca 19-9 is positive in 73% of studied cases, while CEA is positive in 66% of studied cases. On the other hand, alkaline phosphatase values were positive only in 46% of studied cases and showed no high specificity in identifying a specific kind of cancer ,but measured in group with other tumor marker can be helpful in monitoring and treatment for malignant pathologies .Table 7

Result for LDH show valued increased in all study patients but many cancers can raise LDH levels, so LDH may be used as a [tumor marker](#), but at the same time, it is not useful in identifying a specific kind of cancer. Measuring LDH levels can be helpful in monitoring treatment for cancer. Noncancerous conditions that can raise LDH levels include heart failure, hypothyroidism, anemia, and lung or liver disease.

Table 7. Statistical summary of analytical results for second study group of patients.

Statistical parameter	CA 19-9 (U/L)	CEA (ng/mL)	ALP (U/L)	LDH (U/L)
Number of patients	15	15	15	15
Mean value	108	29	284	538
STD	155	52	109	113
$\bar{x} \pm \sigma$	108 ±155	29±52	284±109	538±113
% of positivity (Sensitivity)	73%	66%	46%	100%.

Conclusions: Serum CA 19-9 and CEA levels can be measured by two immunometric assays (ELISA and FIA) in different malignant pathologies. The two immunometric methods showed a positive correlation. These methods may be simultaneously applied in the laboratory, or can be adapted to each other by using of linear regression. Tumor markers Ca 19-9 and CEA can be used for diagnosing specific types of cancer, determining the prognosis in a patient, monitoring the course in a patient while receiving from surgery, radiation or chemotherapy.

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O (Informatics/Education/Clinical chemistry) -4

Prevalence of Anti-Insulin, Anti-IA2, Anti-GAD, Anti-IR Auto-antibodies and Anti-Neu5Gc Antibodies in Diabetic Type I And II Patients

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Introduction: Autoimmunity is a major cause of Diabetes, mainly IDDM. Although, inheritance is recognized, the etiology of the disease is not fully understood and environmental factors are believed to be involved.

Background: A number of auto-antibodies are developed in diabetes type IA but also in diabetes type II. Since, antibodies are identified before the onset of disease; their measurement has been proposed as a prognostic tool. Neu5Gc is a sialic acid synthesized by animals but not humans and birds. It can be incorporated in human cells and can trigger immune response. High concentrations of anti-Neu5Gc were detected by our team in diabetic patients, proposing it as a probable environmental factor. =

Objective: Anti-Insulin/anti-IA2/anti-GAD/anti-IR auto-antibodies and anti-Neu5GcIgG/IgM/IgA antibodies were measured in 40 type I and 50 type II patients. A probable correlation between the development of anti-Neu5Gc antibodies and auto-antibodies is investigated and their prognostic value is discussed.

Results and Discussion: IgG/IgM/IgA auto-antibodies as well as anti-Neu5Gc antibodies were present in 50% of Diabetic type I patients. 88.9% of auto-antibody positive samples were also anti-Neu5Gc positive and 90.9% of auto-antibody negative were also anti-Neu5Gc negative. Anti-insulin/anti-IA2/anti-GAD/anti-IR IgG/IgM/IgA were present in 13.8% of Diabetic type II patients, 83% of which were also anti-Neu5Gc positive.

Conclusion: The results indicate a correlation between development of auto-antibodies and anti-Neu5Gc and introduce anti-Neu5Gc as a probable prognostic factor of diabetes type I.

O (Molecular biology) -1

Prevalence of ARG72PRO polymorphism of tumor suppressor gene p53 in a random sample of general population

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Introduction: The TP53 tumor suppressor gene – also designated the guardian of the genome – encodes p53, the central protein in the apoptotic pathway which has been shown to be of crucial importance in the development of cancers in addition to a variety of neurodegenerative disorders. Predisposition to several human cancers has been associated with genetic polymorphisms, which may represent an important contribution to cancer susceptibility and tumor behavior. One of the most well studied TP53 gene polymorphism is Arg72Pro, located in codon 72 on exon 4, leading to arginine-proline substitution, which in its turn results in a structural alteration of the protein.

Aim of the study: A functionally normal TP53 is essential to protect organisms from developing cancer. The aim of our study was to investigate the prevalence of Arg72Pro polymorphism of TP53 gene, in healthy individuals.

Materials and Methods: Buccal swab and/or oral rinse samples were collected from 83 individuals (60 women, 23 men) at an in person interview, while a written consent and questionnaire were obtained. The mean age of the subjected persons was 22 years (age range 18 to 54). DNA was extracted using the GeneJET Viral DNA and RNA Purification Kit (Thermo scientific, Germany). Analysis of the p53 Arg72Pro genotype was performed by two allelic specific PCRs per sample, as described by Nagpal et al. (2002) with some modifications. Sample collections held at Biology & Genetics Laboratory as well as at Histopathology & Cytopathology Laboratory of Faculty of Medical Laboratory Studies, A.T.E.I.Th.

Results: In cohort of 83 healthy controls from Greek general population, we have shown the distribution of a well-known functional polymorphism in TP53 gene. Four (4.8 %) participants were Pro/Pro (C/C); 41 (49.4 %) were Pro/Arg (C/G), and 38 (45.8%) were Arg/Arg homozygotes (G/G). Frequencies of the Arg (CGC) and Pro (CCC) allele were 0.7 and 0.3 respectively.

Conclusions: Studies show that there is a correlation between SNP: TP53 codon 72 and ethnicity. For example, in Caucasian, Turkish and Japanese population, allele G is more prevalent than C; in Africans, allele G is less than C. The allele frequencies determined in our study are in accordance with previously published allele frequencies in Caucasian populations, although the Arg allele frequency is increased compared with a previous study in Greece (Kalemi T.G. et al, 2005).

O (Molecular biology) -2

Polymorphism 4G/5G of plasminogen activator inhibitor-1 (PAI-1) gene in general population sample of Northern Greece

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Introduction: Plasminogen activator inhibitor type 1 (PAI-1) is an important component of the coagulation system that down-regulates fibrinolysis in the circulation. Reduced PAI-1 levels may result in increased fibrinolysis and an associated bleeding diathesis. A single-base-pair guanine insertion/deletion polymorphism (4G/5G) within the promoter region of the PAI-1 gene has been related to various vascular diseases such as myocardial infarction and deep vein thrombosis as well as to pregnancy-related disorders such as severe pre-eclampsia, pregnancy-induced hypertension, or serious pregnancy complications such as growth retardation and stillbirth. Homozygotes for the deleted allele (4G/4G) carrying the highest plasma levels of this inhibitor.

Aim of the study: The aim of the study was to determine the prevalence of PAI-1 4G/5G polymorphism in a sample of general population originated mostly from Northern Greece.

Materials and Methods: Our study group consisted of 122 individuals (85 women and 37 men) with a mean age of 28 years (range 18–82). The objective of the study was fully explained to all participants and written consent and questionnaire were obtained. Genomic DNA was isolated from blood using QiaAmp DNA Blood Mini kit (Qiagen), according to the manufacturer's protocol. PAI-1 4G/5G promoter genotype was established for each subject

by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis.

Results: In the studied population, the genotype frequencies detected as follow: 31.1% (n=38) for the 4G/4G genotype, 56.6% (n=69) for the heterozygous genotype (4G/5G) and 12.3% (n=15) for the 5G/5G genotype. The calculated allele frequencies were 0.59 for 4G and 0.41 for 5G allele. The genotype frequencies for PAI-1 4G/5G polymorphism were boundary consistent with Hardy–Weinberg equilibrium (p value = 0.056).

Conclusions: The allele frequencies observed are in accordance with those reported in other Caucasian populations (Czech Republic, Germany) as well as in Japan. In contrast, the 5G allele frequency exhibits a higher value than 4G allele compared to our study results, in Chinese, Lebanese or Gaza Strip populations. This report may serve as a baseline for planning further investigations of this polymorphism in association with several clinical entities.

O (Molecular biology) -3

Distribution of insertion /deletion polymorphism of angiotensin I converting enzyme (ACE I/D) in healthy Greek population

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Introduction: Angiotensin II, a very potent vasoconstrictor, is generated by the angiotensin I converting enzyme (ACE), which is well known for its role in blood pressure regulation. ACE expression is associated with a deletion (D)/insertion (I) polymorphism in intron 16 of the ACE gene. This genetic variation is associated with several diseases, such as coronary heart disease, atherosclerosis, acute myocardial infarction and ischemic stroke, diabetes nephropathy, and in the last decade with recurrent spontaneous miscarriages.

Aim of the study: to investigate the ACE I/D genotype frequencies among healthy population of Northern Greece.

Materials and Methods: A total of 117 people (80 women and 37 men) were subjected to the study, with an age range from 18 to 82 (mean=28 years). The majority of blood samples were collected during a voluntary blood donation held at A.T.E.I.Th. To analyse the D/I polymorphism in intron 16 of the ACE gene, genomic DNA was extracted from leukocytes of the individuals and amplified by PCR using –specific primers. The ACE D/I genotype was characterized by the length of the PCR product, 190 bp in the case of the deletion and 490 bp in the presence of the insertion. All samples were collected from participants with their consent and while completing a questionnaire.

Results: The frequencies of the I/I, I/D and D/D genotypes in the study population were 11.8% (n = 14), 37.8% (n = 45), 50.4% (n = 60), respectively. The frequencies of the I and D alleles were 0.31 and 0.69. The genotype frequencies for ACE I/D polymorphism were in Hardy–Weinberg equilibrium (p value = 0.227). Our results are significantly different from the ACE I/D genotype distribution reported in previous population studies related to European countries ($P < 0.05$).

Conclusions: We determined a relatively high percentage DD genotype (50.45) and a high frequency (0.69) for the D allele. The present Greek I/D frequencies are closer to those reported for a Gaza Strip population ($P=0.412$). Large differences between the reported I/D frequencies for European populations have been noticed, so it is difficult to determine average ACE I/D frequencies for the Caucasian populations as a group. Thus, it is not safe to compare I/D frequencies found in study groups from different countries while examining the association of the ACE I/D polymorphism in associated diseases.

O (Molecular biology) -4

Real time-PCR molecular technique and diagnostic applications

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Real-time reverse-transcriptase RT-PCR quantitates the initial amount of the template most specifically, sensitively and reproducibly, and is a preferable alternative to other forms of quantitative RT-PCR that detect the amount of final amplified product at the end-point (Freeman, 1999, Raeymaekers, 2000, Espy, 2006). Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle (in real time) as opposed to the endpoint detection (Higuchi, 1992, Higuchi, 1993). The real-time progress of the reaction can be viewed in some systems.

Real-time PCR does not detect the size of the amplicon and thus does not allow the differentiation between DNA and cDNA amplification, however, it is not influenced by non-specific amplification unless SYBR Green is used. Real-time PCR quantitation (qPCR) eliminates post-PCR processing of PCR products (which is necessary in competitive RT-PCR). This helps to increase throughput and reduce the chances of carryover contamination. In comparison to conventional RT-PCR, real-time PCR also offers a much wider dynamic range of up to 10⁷-fold (compared to 1000-fold in conventional RT-PCR). Dynamic range of any assay determines how much target concentration can vary and still be quantified. A wide dynamic range means that a wide range of ratios of target and normalizer can be assayed with equal sensitivity and specificity. It follows that the broader the dynamic range, the more accurate the quantitation. The real-time PCR system is based on the detection and quantitation of a fluorescent reporter (Lee, 1993, Livak, 1995). This signal increases in direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase, where the first significant increase in the amount of PCR product correlates to the initial amount of target template. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. A significant increase in fluorescence above the baseline value measured during the 3-15 cycles indicates the detection of accumulated PCR product. A fixed fluorescence threshold is set significantly above the baseline that can be altered by the operator. The parameter CT (threshold cycle) is defined as the cycle number at which the fluorescence emission exceeds the fixed threshold. There are three main fluorescence-monitoring systems for DNA amplification (Wittwer, 1997a): (1) hydrolysis probes, (2) hybridizing probes and (3) DNA-binding agents (Wittwer, 1997b, van der Velden, 2003). Hydrolysis probes include TaqMan probes (Heid, 1996), molecular beacons (Mhlanga, 2001, Vet, 2002, Abravaya, 2003, Tan, 2004, Vet & Marras, 2005) and scorpions (Saha, 2001, Solinas, 2001, Terry, 2002). They use the fluorogenic 5' exonuclease activity of Taq polymerase to measure the amount of target sequences in cDNA samples.

All real-time PCR systems rely upon the detection and quantitation of a fluorescent reporter, the signal of which increases in direct proportion to the amount of PCR product in a reaction.

In the simplest and most economical format, that reporter is the double-strand DNA-specific dye SYBR[®] Green (Molecular Probes). SYBR Green binds double-stranded DNA, and upon excitation emits light. Thus, as a PCR product accumulates, fluorescence increases. The advantages of SYBR Green are that it's inexpensive, easy to use, and sensitive. The disadvantage is that SYBR Green will bind to any double-stranded DNA in the reaction, including primer-dimers and other non-specific reaction products, which results in an overestimation of the target concentration. For single PCR product reactions with well designed primers, SYBR Green can work extremely well, with spurious non-specific background only showing up in very late cycles.

The two most popular alternatives to SYBR Green are: TaqMan[®] and molecular beacons, both of which are hybridization probes relying on fluorescence resonance energy transfer (FRET) for quantitation. TaqMan Probes are oligonucleotides that contain a fluorescent dye, typically on the 5' base, and a quenching dye, typically located on the 3' base. When irradiated, the excited fluorescent dye transfers energy to the nearby quenching dye molecule rather than fluorescing, resulting in a nonfluorescent substrate. TaqMan probes are designed to hybridize to an internal region of a PCR product. During PCR, when the polymerase replicates a template on which a TaqMan probe is bound, the 5' exonuclease activity of the polymerase

cleaves the probe. This separates the fluorescent and quenching dyes and FRET no longer occurs. Fluorescence increases in each cycle, proportional to the rate of probe cleavage. Molecular beacons also contain fluorescent and quenching dyes, but FRET only occurs, when the quenching dye is directly adjacent to the fluorescent dye. Molecular beacons are designed to adopt a hairpin structure while free in solution, bringing the fluorescent dye and quencher in close proximity. When a molecular beacon hybridizes to a target, the fluorescent dye and quencher are separated, FRET does not occur, and the fluorescent dye emits light upon irradiation. Unlike TaqMan probes, molecular beacons are designed to remain intact during the amplification reaction, and must rebind to target in every cycle for signal measurement.

(<http://qpcr.gene-quantification.info/>). There are several applications for quantitative polymerase chain reaction in the laboratory. It is commonly used for both diagnostic and basic research. Uses of the technique in industry include the quantification of microbial load in foods or on vegetable matter, the detection of GMOs (Genetically modified organisms) and the quantification and genotyping of human viral pathogens. Diagnostic quantitative PCR is applied to rapidly detect nucleic acids that are diagnostic of, for example, infectious diseases, cancer and genetic abnormalities. The introduction of quantitative PCR assays to the clinical microbiology laboratory has significantly improved the diagnosis of infectious diseases, and is deployed as a tool to detect newly emerging diseases, such as new strains of flu, in diagnostic tests.

Model of real time quantitative PCR plot

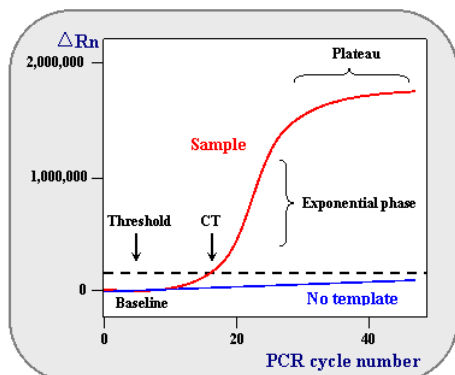


Figure 1. Model of real time quantitative PCR plot

Nomenclature commonly used in real time quantitative RT-PCR:

Baseline: is defined as PCR cycles in which a reporter fluorescent signal is accumulating but is beneath the limits of detection of the instrument. ΔRn is an increment of fluorescent signal at each time point. The ΔRn values are plotted versus the cycle number.

Threshold: is an arbitrary level of fluorescence chosen on the basis of the baseline variability. A signal that is detected above the threshold is considered a real signal that can be used to define the threshold cycle (Ct) for a sample. Threshold can be adjusted for each experiment so that it is in the region of exponential amplification across all plots. **Ct:** is defined as the fractional PCR cycle number at which the reporter fluorescence is greater than the threshold. The Ct is a basic principle of real time PCR and is an essential component in producing accurate and reproducible data.

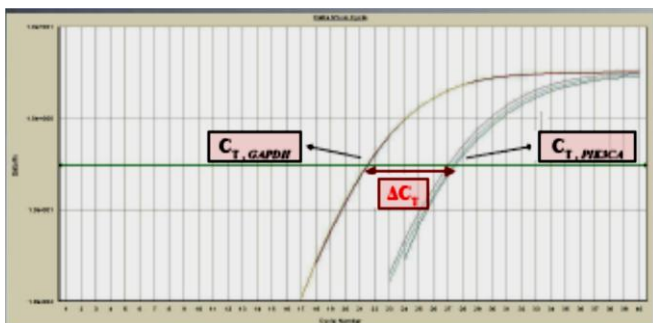


Figure 2: Amplification plots of *PIK3CA* and *GAPDH* cDNAs. *PIK3CA* mRNA expression was detected by real-time quantitative PCR, using the comparative $CT(2^{-\Delta\Delta C_T})$ method. The software constructs amplification plots, where ΔRn is plotted versus cycle number. *PIK3CA* mRNA copies/*GAPDH* mRNA copies are calculated using the formula: $2^{-\Delta C_T}$. Real-time PCR was carried out in an Applied Biosystems 7500 Real Time PCR System. The dye we used was SYBR Green as molecular probe (Papalexis P. et al. 2010).

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O (Molecular biology) -5

Humans, *C. elegans* and DNA: a worm's tale.

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Caenorhabditis elegans is a ubiquitous saprophytic nematode species inhabiting soil and leaf litter environments nearly worldwide (Hope, 1999). It appeared in the literature as early as the beginning of last century (Maupus, 1900), but it was the publication of Sydney Brenner's seminal paper (Brenner, 1974) that landmarked its significance as an experimental animal model.

This nematode has a number of characteristics that make it an outstanding candidate as a model for biological research. It is a simple multicellular animal, easy and inexpensive to grow in the laboratory, and with a short life cycle (3 days per generation). Its small body size makes it amenable to *in vivo* studies in 96-well micro plates and to high throughput screening. Its transparent body allows detailed observation of all its cells, at all stages of development and during cell death. Embryonic (Sulston *et al.*, 1983) and post embryonic (Sulston and Horvitz, 1977) development cell lineages have been established and transgenic reporters (eg GFP) can be used to identify specific cell features, as well as localized gene expression, leading to fully described anatomy and development. In addition, *C. elegans* is invariant and eutelic, and therefore its neuroanatomy is also invariant, allowing the reconstruction of its nervous system (Ward *et al.*, 1975). Its powerful genetics (both self-fertile and cross-fertile), the existence of well-defined mutants, as well as being the first multicellular organism whose genome was sequenced (*C. elegans* sequencing Consortium, 1998), enhanced further its status as a model biological system of choice.

Currently, there are post-genomic tools available in *C. elegans* research, continuing the annotation of its genome, initiating EST projects to define mRNA and splice variants, enabling systematic gene expression studies and whole genome microarray experiments, while the sequencing of other caenorhabditid species is in progress. Systematic gene knockouts by deletion screening and RNAi (Johnson *et al.*, 2005), and proteomic analyses are also being implemented, while novel technologies, such as manipulation of gene expression, worm-flow cytometry, microfluidics, imaging and optogenetics (Xu and Kim, 2011) have been developed to enhance the *C. elegans* tool kit. Moreover, research in the areas of cell death, developmental signaling, endocrine signaling, stress responses, metabolism and miRNAs, has benefitted by the utilization of *C. elegans* as a general research tool.

The worm has also become a premier model organism to study longevity and the aging process. Aging research has advanced greatly in the nematode over the past 20 years, and currently distinct pathways that impinge in the aging process are being pieced together. Endocrine signaling has a key role in most of these pathways, including the insulin/insulin-like growth factor (IGF-1) signaling pathway (IIS) (Friedman and Johnson, 1988; Kenyon *et al.*, 1993) and the germline signaling pathway (Hsin and Kenyon, 1999). These findings are important steps in the understanding of the aging process not only in *C. elegans* but in other multicellular organisms (Panowski and Dillin, 2009).

Caenorhabditis elegans is an important organism for genetic studies also in the context of human disease. For example, Briese *et al.* (2009) found that deletion of a *C. elegans* ortholog to the spinal muscular atrophy gene causes locomotor dysfunction and reduced lifespan. In another study it was shown that APL-1, the Alzheimer's amyloid precursor protein in *C. elegans*, modulates multiple metabolic pathways throughout development (Elwad *et al.*, 2012). In this wider context, efforts have been initiated recently to assess the orthology between human and *C. elegans* genes, so that RNAi screens will be streamlined by focusing on genes with human orthologs and therefore reducing screening efforts by approximately 60% (Shaye and Greenwald, 2011). The *C. elegans* gene *bus-8* plays a role in epidermal morphogenesis and encodes a glycotransferase (T23F2.1) homologous to mannosyltransferases involved in protein N-glycosylation (Partridge *et al.*, 2008). Understanding the role of glycosylation in the development of model organisms and in effect in human developmental disorders has considerable therapeutic potential.

Host pathogen-interactions constitute another area where this nematode is of significance. This type of research aims to elucidate the number of genes involved in biotic interactions such as antimicrobial

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defense in *C. elegans*, how the nematodes detect and combat microbial infection and how their immunity is integrated with development. Microbial virulence has been modeled in *C. elegans* (Sifri *et al.*, 2005), distinct phenotypes due to microbial infection have been documented (Hodgkin *et al.*, 2000; Nicholas and Hodgkin, 2004) and mutants resistant to infection have been established (Darby *et al.*, 2002; Gravato-Nobre and Hodgkin, 2008). In addition, the molecular mechanisms involved in the establishment of microbial infection in *C. elegans* have begun being unraveled (O'Rourke *et al.*, 2006), while recent work (Félix *et al.*, 2011) has started paving the way to understand nematode-virus interactions and the host mechanisms that counter viral infection.

The same features that have made *C. elegans* an attractive model organism in the other areas of biological science, have also led to the increasing use of *C. elegans* in toxicology, particularly in biomedical and environmental toxicology, with emphasis on neurotoxicity, neurodegeneration and DNA damage (Leung *et al.*, 2008). At the same time, transgenic *C. elegans* biosensors are available (Lagido *et al.*, 2001) for the monitoring of toxicants in the environment.

The attention that this nematode has received is so vast and its applications so multiple and varied that a plethora of resources is available (Antoshechkin and Stenberg, 2007) that make this system even more attractive and thus paving the way for numerous future novel investigations. The "Million Mutation Project" (Thompson *et al.*, 2013) is an example of an ambitious and unprecedented effort to build a genetic resource for this multicellular organism, and it clearly demonstrates the immense and to be explored potential of this humble and yet so influential animal.

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P (Clinical chemistry) -1

AQt90 Analyzer as a Point of Care

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Introduction: A lot of interest toward point-of-care testing for biochemical markers of myocardial cell necrosis in addition to traditional testing is found in clinical field. Since this technology introduced a wide range of tests to be performed simply and quickly.

However, rapid patient triage, decreased length of stay and improvement in turnaround time, lack of precision, sensitivity, increased cost and no demonstration in improvement in patient outcomes gave a mixed opinion regarding the issue of POC that precludes their routine use and recommendation.

We tested AQt90 analyzer / Radiometer as a random access immunoassay system "whole blood" for patients with suspected cardiac symptoms "Troponin I, CKMB, Myoglobin, and NT- pro BNP and D-Dimer".

Method: blood samples from suspected cardiac patients or coagulation abnormalities were collected using EDTA as standard sampling tube. Citrated tube was used for D-Dimer to be compared with latex agglutination method" DIMERTEST, American Diagnostica" and EDTA samples. Comparison with Beckman access for Troponin I was also done.

Results: 147 patients "75 males & 72 females" were involved randomly. Citrated and EDTA D-Dimer tests correlated significantly $P = 0.0001$. D-Dimer tests 1000 ng/ml showed positive results with latex agglutination. Interestingly, D-Dimer correlated with NT-pro BNP, $p = 0.001$. Troponin correlated with CKMB $p = 0.035$, NT-pro BNP $p = 0.03$ but not Myoglobin $p = 0.4$ or D-Dimer $p = 0.9$. Troponin I (median_{AQt90} 0 ng/ml), however, failed to show correlation with Beckman access (median_{Access} 0.01 ng/ml).

Conclusion: AQt90 could be a valuable POC testing in addition to traditional testing for cardiac patients using EDTA samples only. More samples needed for Troponin comparison.

P (Clinical chemistry) -2

Frequency of Hypomagnesemia in Patients with Type 2 Diabetes Mellitus

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Magnesium is an important factor in a variety of cellular processes related to glucose metabolism and insulin secretion and activity. Hypomagnesemia is observed at an increased frequency among patients with type 2 diabetes mellitus (DM) and is linked to poor glycemic control. The aim of this study was to evaluate the frequency of hypomagnesemia in type 2 DM patients.

The study included 1200 patients, 680 men and 580 women aged between 32 and 70 years and 200 age-matched healthy controls, 139 men and 62 women. Levels of serum magnesium (Mg) and fasting glucose levels (GLU) were measured on the Olympus AU640 fully automated analyzer, using the commercial kits PN 1418-0250 and 1418-0013 respectively, by Medicon Hellas SA. The normal serum Mg level was considered as 1.8 to 2.8 mg/dl. Data were analyzed with linear regression analysis and differences between patients and healthy controls, as well as between men and women were evaluated by Student t-test (p value = 0.05).

407(33.9%) patients, 236 men and 171 women, presented hypomagnesemia with serum Mg levels ranging from 0.48 to 1.79 mg/dl (mean value 1.56 mg/dl). Patients had significantly lower serum Mg levels than healthy controls. Statistically significant differences between men and women were also observed. Moreover, we observed significant inverse correlation between Mg and GLU levels ($r=-0.835$) in patients with hypomagnesemia.

Hypomagnesemia in 1patients with type 2 DM and is mainly attributed to increased urinary loss, lower dietary intake, or impaired absorption of Mg compared to healthy individuals. In the present study hypomagnesemia was observed in 33.9% of DM patients. The wide range in the reported frequencies may reflect different study designs and heterogeneity of the selected populations. Our results also revealed a statistically significant difference between men and women for p values ≥ 0.05 .

P (Clinical chemistry) -3

Cardiac Markers, New Definition and Reality

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Background: It's almost now five years since the ACC/ESC committee announced their new definition of myocardial infarction that includes troponin as best available cardiac marker. Still some hospital retaining their old way mainly WHO criteria for their use of laboratory cardiac markers. Despite the availability of that gold standard, cardiologist still retain the old way of serial measurement of cardiac markers with less dependant on that marker. Is it a matter of lack of confidence in troponin, an opinion, or the refuse to change is still unknown?

Method: Cardiac markers for 196 (74 females, 122 males) patients wererequested byphysicians were involved in this case. Results were recorded, studied and compared using analyze it statistical package.

Results: Fortunately, troponin; median 1.4 ng/ml was ordered for only three patients with almost complete dependant on old cardiac markers. AST: median 26 u/L (I.Q.R 19.5-35), CK: median 124 u/L (I.Q.R 86-178), CK_{activity}: median 4 U/L (I.Q.R 34-63), and LDL: median 147 U/L (I.Q.R 129.5-189). Using CK and CKM_{Bactivity} significant difference were found between males and females (p 0.001, p = 0.03 respectively) while others didn't show any sex difference. All markers showed significant linear correlation (p 0.001).

Conclusion: Body mass should be taken in consideration when dealing with some old cardiac markers.

P (Clinical chemistry) -4

Zinc Deficiency

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Introduction: Zinc is an essential mineral that is required for every system in our body during different stages of life. Although, deficiency of zinc usually manifested by delayed healing of wounds, growth and mental retardation, taste abnormalities and others, loss of hair constitutes the main complain especially among females. Seeking medical advice for this problem would be by identifying reasons for this deficiency before direct diagnosis of zinc deficiency with prescription of zinc as the final decision.

Method: In this study we have revised zinc and other tests results for 105 patients (10 males, 95 females) attended military hospital in the period of 4 years.

Result: Zinc 12.9 nmol/l (IQR 11.200-16.000) and positively skewed curve with $p = 0.001$. Man-Whitney test showed significant difference in the medians between females (median 12.48 nmol/l; IQR 10.95-13.45) and males (median 18.13 nmol/l; IQR 13.64-20.95). However, none of the patients in this study have other tests done for them such as thyroid function test, iron, or fluorides.

Conclusion: Identifying underlying causes and precipitating factor is important in trace elements deficiency especially in females.

P (Clinical chemistry) -5

Development of an Assay for Detecting α -2- Macroglobulin

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Introduction: Diagnosis of kidney problem is usually made depending on clinical history, medical examination, X-ray and laboratory tests. One of these laboratory tests is the presence of blood (haematuria) and proteins (proteinuria) in urine. Proteins especially the one with large size, are reabsorbed in the urinary tract and don't present in urine of healthy people except when there primary or secondary kidney problems. We tried to develop an ELISA assay for the measurement of α -2- macroglobulin in urine and evaluate its performance. Testing α -2- macroglobulin as marker for haematuria.

Method: Method was established using commercial reagents , equipment and appropriate standards. Standard prepared by adding 0.2ml plasma calibrator to 2ml of 1% BSA/PBS to give concentration of 181.8 mg/l α -2- macroglobulin . Serial dilution of the curve was done to give a range from 0.04 μ g/l -874 g/l. The assay was optimized using different regants and conditions.

Urine samples from patients with kidney problems were tested for α -2- macroglobulin. The results were compared to blood content measured using urine strips. All results were tested statistically using analyze-it.

Result: Standard curve constructed using serial measurement of the standard with optimum conjugated antibody concentration 0.049 μ g/ml and 14.2 μ g/ml for coating antibody. Ova-albumin was chosen as a blocker. Sensitivity of the assay was 0.05 μ g/ml, analytical recovery 86-110%. Dilution experiments showed parallism in standard curves. Intra and inter- assay were 1.9-5.1% and 9.9-20% respectively. α -2- macroglobulin measured using the assay correlated with haematuria (p 0.05; Spearman correlation) and proteinuria (p 0.01). α -2- macroglobulin correlated with proteinuria in those who were positive for haematuria p 0.01).

Conclusion: Positive correlation with proteinuria excludes α -2-macroglobulinn from being used as a sole marker for haematuria.

P (Clinical chemistry) -6

A Study in Total Vitamin D Assay

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Background: A renewed interest in vitamin D (vt.D) reflects almost the high prevalence of vitamin D deficiency worldwide and the increased publication connecting its deficiency to other clinical conditions than bone health. In most assays underestimation of the vitamin levels observed even with the new measurement of total vitamin D (D₃+ D₂) assay.

Method: we tested total vitamin D results in randomly selected 243 patients attending J.A. armed forces hospital. We compared vt.D assay for samples at our hospital (LIAISON, diaSorin) and (ROCHE, COBAS 6000 in MOH hospital). We studied the link between total vt. D, serum calcium, parathyroid hormone, glucose and hba_{1c}.

Results: 41 males & 202 females were involved with descriptive statistics shown in table.1. No statistically significant difference was found in vt D concentration between both sexes (Mann-Whitney P = 0.6. LIAISON & COBAS vt.D results correlated significantly (P = 0.001, R² = 0.87; linear regression). No Correlations (spearman) were found between vt.D & ca (P = 0.9), PTH (P = 0.4), Glucose (P = 0.6) or hba_{1c} (P = 0.2).

Conclusion: measurement of total vt.D provides crude assessment its status but may give inaccurate indication of its biological activity. Further studies needed

	Vt.D nmol/l	Calcium mmol/l	PTH pmol/l	Glucose mmol/l	Hba1c %
median	22.3	2.3	26.4	5.4	7.1
25 % I.Q.R	19.03	2.2	17.8	5	6.2
75% I.Q.R	58.1	2.4	60.5	6.3	9.1

P (Clinical chemistry) -7

Synthesis and Spectroscopic study of Acetyl Taurine-Calcium salt. A potential therapeutic agent.

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Introduction: Taurine is a conditionally-essential amino acid that may be called a sulphonic amino acid. Taurine is in high abundance in many tissues, has numerous physiological and pharmacological actions and possesses a broad spectrum of biological and metabolic functions

Because of its therapeutic possibilities, taurine has been used with a different degree of success in the treatment of a variety of pathological conditions.

On the other hand, Ca²⁺ ions belong to the extracellular ions and are an effective trigger for the activation of many biological reactions. One of the most important roles of calcium in advanced biological systems is its involvement in metabolism.

Purpose: The synthesis of taurine salt with Ca²⁺ cations has been studied by means of spectroscopic methods (FT-IR and ¹HNMR) in order to combine the actions of taurine with those of the metal ions and to create a more effective therapeutic agent.

Materials and Methods: Materials Taurine, C₂H₇NO₃S, was obtained from Sigma as a white crystalline compound of minimum purity 99 %. Calcium Hydroxide, Ca(OH)₂, of > 98 % purity was purchased from Acros. These reagents were used without further purification.

Acetic anhydride, C₄H₆O₃, was obtained from Riedel-de Haën and it was of 98% purity. Methanol with upgraded quality was obtained from Fluka.

Physical methods

- Fourier Transform Infrared Spectroscopy, FT-IR
- Nuclear Magnetic Resonance Spectroscopy, ¹HNMR
- Atom absorption Spectroscopy, AAS

Results and Discussion: The FT-IR spectra of taurine before and after interaction with calcium cations showed considerable changes of the characteristic vibrational bands of sulphonic group leading to suggestion that Ca²⁺ binds to oxygen atom of the sulphonic group of acetyl taurine. The new absorption bands in the region 4000-3000 cm⁻¹ were attributed to symmetric and asymmetric stretching vibrations of νOH, νNH, suggesting the binding of Ca²⁺ cations. The characteristic bands of SO₃⁻ groups shifted to lower wavenumbers after the reaction, concerning the binding of the calcium cations.

¹HNMR spectra showed that the signals of C(1)H and C(2)H shifted to lower and higher δ values, respectively upon the reaction.

¹HNMR spectra of the acetyl taurine salt proves the presence of acetyl taurine in the new molecule and shows that the binding with the metal ions is realized through the sulphonic group.

Chemical element analysis and AAS analysis showed that the Ca²⁺ cations interacted with taurine in a ratio of 1:2 and general chemical molecular formula C₈H₁₆O₈N₂S₂Ca·4H₂O.

Conclusions: It is concluded from this study that the ligand acetyl taurine reacts with calcium ions to form a salt of the formula, CaL₂·4H₂O (L= C₄H₈O₄NS).

Acetyl taurine binds to calcium ions through the O atom of its sulphonic group acting as a monodentate ligand.

This research shows that metal ions move in body fluids as complexes or metal salts, combined with biological molecules, like in our case with taurine.

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P (Clinical chemistry) -8

Matrix Metalloproteinase-1 and Tissue Inhibitors-1 and -2 in Obesity

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Introduction: Obesity is a chronic disease that keeps increasing in industrialized countries and now on the rise in low - and middle - income countries.

Matrix metalloproteinases (MMPs) are proteolytic enzymes capable of extracellular matrix remodeling. MMP-1 is a collagenase which deregulation is reported in many inflammatory diseases. MMPs and their tissue inhibitors (TIMPs) are associated with adipose tissue modification during the development of obesity. TIMP-1 is described as an adipokine and TIMP-2 exerts a role in maintaining a normal adipocyte physiology. Consequently, is expected an upregulation of its secretion in obesity.

Aims: To compare serum and saliva levels of MMP-1 and tissue inhibitors TIMP-1 and TIMP-2 in normal weight, overweight and obese young adults. Additionally, correlate both biological fluids.

Design and Methods: This study included analyses of serum and saliva samples from 72 individuals classified by body fat percentage and divided in 3 groups: normal weight, overweight and obese. Correlation between the two fluids was also performed. All samples were analyzed using slot blot technique.

Results: The data showed that MMP-1 serum and saliva levels tended to be lower in individuals which have higher percentage of body fat compared to normal weight, in spite of the fact that these results did not differ significantly. Significant differences were observed in TIMP-1 measured in saliva between the group's normal weight and overweight. A moderate negative correlation between saliva and serum levels of TIMP-1 was also evidenced in this study.

Discussion and Conclusion: Data of this study suggest that TIMP-1 may be involved in physiopathological mechanisms of obesity. Given the results of MMP-1, new investigations should be conducted in a population with more years of obesity to disclose the influence of this enzyme in this disease. The results also highlight the potential use of saliva to monitor and early diagnose obesity-related complications.

P (Clinical chemistry) -9

Cholinesterase Activity in Alzheimer's Disease

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Introduction: Alzheimer's disease (AD) is a complex brain disorder that occurs in a progressive deterioration of memory along with other cognitive domains. This is a growing public health problem, and it is estimated that in 2050, approximately 115 million people will suffer from this dementia worldwide. The β -amyloid peptide ($A\beta_{1-42}$), total tau protein (T-tau) and hyperphosphorylated tau (P-tau₁₈₁) are used as biomarkers of AD, reflecting their main histopathologic characteristics.

Are also evidenced changes in the cholinergic system. In this sense it is important to evaluate the activity of cholinesterase (ChE), acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE), these may be related with reduced reduced brain activity of the neurotransmitter acetylcholine (ACh), reflecting changes in learning, memory and cognitive processes, characteristic of this dementia.

Aims: Evaluate the activity of AChE and BuChE in cerebrospinal fluid (CSF) of patients with AD and mild cognitive impairment (MCI). Discuss how the activity of ChE influence on impairment of the cholinergic system in various pathological conditions, and how the interactions of $A\beta_{1-42}$, T-tau and P-tau₁₈₁, influence synaptic function and neurodegeneration in AD patients.

Material and Methods: Were studied 48 CSF samples from patients with AD and MCI, relatively to the activity of AChE, BuChE, by the method of Arrudaet al.

Results: It was observed that of AChE activity is decreased in patients with MCI, compared to AD patients. Was demonstrated significant correlation between the values of AChE and BuChE in both pathological conditions?

Discussion and Conclusion: The evaluation of AChE in subjects with MCI may have a predictive value in regard to this transition condition for AD pathology. The lower values of AChE may reflect a greater BuChE activity in subjects with MCI. In patients with AD, increased activity of AChE and BuChE are c concomitantly connected.

P (Clinical chemistry) -10

Evaluation of Protein Oxidation and VEGF levels in Obesity

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Introduction: Obesity is a chronic disease characterized by excessive accumulation of fat in adipose tissue. It represents a major global health challenge, due to the increasing prevalence and high morbidity and mortality caused by several associated complications. The inflammation in obesity results from an increase of adipose tissue, hypertrophy and hyperplasia of its cells and is associated with immune system dysfunction and increased oxidative stress. With the increased number of adipocytes and macrophages, which have secretory activity, there is an increased production of proinflammatory mediators such as tumor necrosis factor- α (TNF- α), vascular endothelial growth factor (VEGF), among others. VEGF stimulates angiogenesis and neovascularization, resulting in the growth of new blood vessels in adipose tissue. Other adipokines have the characteristic to generate the release of reactive species which have the capacity to damage different cellular components, particularly proteins, leading to several physiopathological processes, such as diabetes, hypertension, heart disease and some types of cancers.

Aim: Evaluation of protein oxidation in obesity by determining serum and salivary protein carbonyls and VEGF levels, and comparison of results between three groups of young: normal weight, overweight and obese.

Material and Methods: A semi-quantification of protein carbonyls and VEGF was performed using the slot blot technique.

Results: Those with higher percentages of fat mass have higher levels of VEGF and protein oxidation than the individuals in normal weight group, both in serum or saliva. However, the results obtained in saliva showed the most marked differences between groups.

Discussion and Conclusion: The levels of protein oxidation and VEGF are increased in obesity and may play an active role in the physiopathology. Saliva has proved to be a fluid with huge potential in the early diagnosis that can be used in future as an alternative to serum.

Keywords: Obesity, Inflammation, VEGF; Protein Oxidation; Saliva.

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P (Clinical chemistry) -11

Effects of Acute Cigarette Smoking on Serum Vitamin E and MDA Levels

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Introduction: Cigarette smoke has been demonstrated to contain a variety of xenobiotics, 26% stay in the filter, 28% are inhaled by the smoker and 46% are dispersed in the air. Cigarette smoking leads to oxygen free radical formation and is involved in the development of serious pathological conditions

Background: Acute smoking causes endothelial dysfunction through impairment of nitric oxide (NO) production, or increased oxidative stress, but the exact mechanism still needs to be elucidated. In healthy non-smokers acute endothelial dysfunction caused by smoking one cigarette was counterbalanced by antioxidants. Oxidative stress is assessed indirectly by the measurement of the secondary products, such as malondialdehyde (MDA).

Objective: The aim of the present study is to investigate how the blood levels of antioxidant vitamin E, and MDA are altered after acute smoking in healthy and passive smokers.

Material and Methods: Serum MDA and Vitamin E was determined in 30 healthy volunteers, 15 smokers (Group A) and 15 non smokers (Group B) before and after acute smoking. All volunteers had no food, drink, or cigarette for 8 hours before the study. MDA was determined by thiobarbituric acid reactive substances assay (TBRAS) method and Vitamin E by reverse phase high performance liquid chromatography. Statistical analysis was done by t test using SPSS. The $p < 0.05$ was considered statistically significant.

Results and Discussion: Results demonstrated that there was a significant increase in MDA ($p < 0.005$) after acute smoking in both groups of active, $p = 0.004$, and passive, $p = 0.000$, smokers and a significant decrease in vitamin E ($p = 0.001$ in active and $p = 0.000$ in passive smokers respectively). The increase in MDA serum levels after acute smoking was more than 100% in both groups.

Conclusion: The increase in serum MDA level and decrease in vitamin E was found in both groups after acute smoking. Vitamin E supplementation is recommended in smokers, or passive smokers, to prevent oxidative stress.

P (Clinical chemistry) -12

Serum Osteoprotegerin in Relation with the Risk of Vascular Disease in Type 2 Diabetic Postmenopausal Women

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Introduction: Osteoprotegerin (OPG) is a plasma glycoprotein produced in many different tissues. It was first known as a key regulator in bone turnover.

Background: Many evidences have demonstrated a relationship between bone pathology and cardiovascular disease (CVD). It is well established that patients with diabetes have a higher risk for CVD.

Objective: To assess serum level of OPG in type 2 diabetic postmenopausal women and studying its association with certain measures of vascular disease and some clinical and biochemical risk factors for vascular disease.

Material and methods: 84 postmenopausal women with type 2 diabetes but without CVD were evaluated for the presence of vascular disease by measuring H-value, an index of arterial pulse wave velocity (PWV) or arterial Hardness in addition to measurement of carotid intima-media thickness (CIMT) and ankle-brachial index (ABI) as noninvasive measures of vascular disease. Their serum OPG level together with some biochemical and inflammatory markers were estimated and certain cardiovascular risk factors were recorded.

Results: Serum OPG level was significantly positively correlated with arterial hardness (H-value) ($r = 0.222$, $P < 0.05$). H-value by itself showed a significant positive correlation with age, systolic pressure, CIMT and ABI. Comparison of OPG levels according to H-value categories (normal, mildly hard and hard arteries) revealed a significant difference in OPG level between normal vs. hard categories (440.88 ± 102.82 vs. 545.51 ± 159.83 ng/L, $P < 0.006$) and between mild vs. hard categories (464.49 ± 100.07 vs. 545.51 ± 159.83 ng/L, $P < 0.029$) while no significant difference was found between normal vs. mild categories (440.88 ± 102.82 vs. 464.49 ± 100.07 ng/L, $P < 0.452$). OPG showed no significant correlation with biochemical or inflammatory parameters except a significant positive correlation with serum triglycerides in dyslipidemic subgroup of patients.

Conclusion: Serum OPG level is significantly associated with arterial hardness as measured by H-value. The highly significant correlations of H-value itself with other measures of vascular disease may refer to an association between OPG and vascular disease in postmenopausal diabetic women and that serum OPG level may be of benefit for risk stratification of such patients.

P (Clinical chemistry) -13

Extended Use of Mobile Phones affect Cortisol and Melatonin Levels

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Background: The health effects of mobile phones have been investigated for many years. Their use has been connected with sleep disorders, mood, and cognition disturbances, hearing loss etc. However, the results are confusing, mainly because of the many factors associated with these disorders.

Introduction: Melatonin was among the biomarkers determined by many scientists. Stable total melatonin production and reduced evening melatonin levels following extended mobile phone use have been recorded. Increased morning (9.00–10.00) melatonin levels were detected in a previous study by our team in the saliva of young strong mobile phone users. Noon melatonin levels were practically the same indicating a gliding of night melatonin curve towards the morning hours. Melatonin regulates many biological processes while its synthesis is subjected to day/light regulation through the suprachiasmatic nuclei (SCN). Cortisol is also regulated through SCN and is the main organism's response to stress factors.

Objective: In the present study, we determined cortisol levels of 32 young volunteers separated into two groups of no/low and extended mobile phone use.

Material and Methods: Saliva collection was done at times 9.00-10.00 and 11.30-13.30. Information about mobile phone & PC use, duration of sleep, awakening time, etc. was gathered. Melatonin and cortisol were determined using colorimetric and luminescence Immunoassays (IBL) respectively.

Results and Discussion: Increased morning cortisol levels ranging from 355-1259ng/dL were observed in the group of extended mobile phone users (257% increase, $p=0.050$). No considerable difference between the two groups was observed at the noon-sampling. In an analogue way, morning melatonin levels were 357% increased ($p=0.007$) in the group of extended mobile phone users.

Conclusion: The results indicate an influence of extended mobile phone use in morning melatonin and cortisol levels. This may have to be taken into account when these parameters or biomarkers related to them are evaluated.

P (Clinical chemistry) -14

Development of LC-MS/MS Method for Determination of 11 β -hydroxysteroid Dehydrogenase Activity in Infertile Men

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Introduction: 11 β -hydroxysteroid dehydrogenase (11 β HSD) is an important enzyme in steroid metabolism that maintains intracellular levels of glucocorticoids. It has two distinct isoforms.

Background: The role of 11 β HSD in testes is conversion of active stress hormone cortisol into inactive cortisone and thus protection of the target tissue from excess of glucocorticoids. Other competitors on active site of 11 β HSD are 7-hydroxylated metabolites of dehydroepiandrosteron (DHEA). Detection of 11 β HSD activity in men with different degree of infertility may contribute to infertility treatment.

Objective: The aim of the study was to develop the LC-MS/MS method for the estimation of cortisol, cortisone, DHEA, 7 α -OH-DHEA, 7 β -OH-DHEA and 7-oxo-DHEA in human sera, calculate cortisol/cortisone ratio and evaluate 11 β HSD activity in lightly and moderately infertile men and in healthy controls.

Material and methods: Two ionization techniques APCI and ESI were tested and their parameters were optimized. Subsequently the parameters of MS/MS detector for individual analytes were optimized. Furthermore, derivatization step at ketogroup was carried out. The Amplifex[®] keto reagent kit and 2-hydrazinopyridine were examined. Also the chromatographic conditions (column, flow, temperature) and mobile phases (methanol, acetonitrile, water with and without formic acid) were tested. Different extraction agents were tried out (diethyl ether, methyl tert-butyl ether).

Results and discussion: After optimization of all parameters we can measure all above mentioned steroids from 500 μ l of serum. The method was used for measuring the steroids in men with different degree of infertility. There was a trend of decreasing activity of 11 β HSD towards infertile men.

Conclusion: The developed method enables rapid and accurate determination of activity of 11 β HSD in serum. The advantage of this method is the simultaneous determination of steroid hormones related to the activity of the enzyme from the small quantities of sample.

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P (Clinical chemistry) -15

The Estimation of 11 β -Hydroxysteroid Dehydrogenase Activity in Patients with Hydrocephalus by LC-MS/MS

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Introduction: 11 β -hydroxysteroid dehydrogenase (11 β -HSD) is tissue-specific enzyme transforming cell deposited inactive cortisone to active cortisol. It catalyses also the conversion of a 7-hydroxylated metabolites of dehydroepiandrosterone (DHEA), forming a competitive reaction to the pair cortisol – cortisone.

Background: Cortisol is an important regulator of inner milieu of brain ventricles. Among others, it may compete for mineralocorticoid receptors present in the brain and thus influence osmotic gradient. Hydrocephalus is a disease characterized by excessive amount of cerebrospinal fluid resulting in the elevation of intracranial pressure and compression of brain structures. The 11 β -HSD activity may be convenient marker for target detection and treatment of hydrocephalus.

Objective: The aim of our study was to develop the LC-MS/MS method for the estimation of cortisol, cortisone, DHEA, 7 α -OH-DHEA, 7 β -OH-DHEA and 7-oxo-DHEA in human sera. Using this method, we measured the levels of abovementioned analytes in serum samples from patients with suspected hydrocephalus.

Materials and methods: 500 μ L of human serum was extracted with diethylether and derivatized with 2-hydrazinopyridine. Sample was dissolved in 5 mmol ammonium formate in 50% methanol, separated by UHPLC and subsequently analysed at API 3200 LC-MS/MS system. Together with the samples, standard curve was analysed, as internal standard D3-DHEA and D4-Cortisol were used.

Results and discussion: The levels of 7 α -OH-DHEA, 7 β -OH-DHEA and 7-oxo-DHEA were statistical significantly elevated in patients with confirmed hydrocephalus compared to the control group of patients. On the basis of our results, we could recommend these steroids as convenient biomarkers for the neurosurgeon's decisions concerning the early and accurate treatment of hydrocephalus.

Conclusion: New developed LC-MS/MS method enables rapid and accurate determination of activity of 11 β -HSD in human serum. The estimation of 11 β -HSD in patients with hydrocephalus could be helpful in indication to surgical intervention leading to treatment of this disease.

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P (Clinical chemistry) -16

Protein Z Levels in Pregnant Women Receiving Low Molecular Weight Heparin due to Hypercoagulability

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Introduction: Protein Z (PZ), a 62Kd vitamin-K dependent plasma glycoprotein serves as a cofactor for the inhibition of coagulation factor Xa by the Z-dependent protease inhibitor (ZPI). The PZ-ZPI complex therefore acts as a naturally occurring anticoagulant. PZ deficiency may predispose to adverse pregnancy outcome. Protein Z increases during normal pregnancy.

Objective: To evaluate PZ levels during pregnancies under anticoagulation treatment affected by thromboembolic complications.

Material and Methods: PZ levels were measured by ELISA in plasma of 32 pregnant women (mean age: 34years, range: 27-44years) receiving Low Molecular Weight Heparin (LMWH) to counteract their hypercoagulability and associated complications (Fetal Growth Retardation, e.t.c.). PZ levels were assessed during the 1st and 3rd trimester of pregnancy.

Results and Discussion: All women had normal PZ levels (1-4 µg/mL) both on their first and last visit, but one was characterized by PZ deficiency, PZ however reached normal values by the 3rd trimester. Six out of 32 women (18.8%) had PZ levels below 2µg/mL in the first trimester, 9 (28.1%) between 2 and 3µg/mL and 16 (50%) over 3µg/mL. The mean PZ levels in women in the 1st and 3rd trimester were 3.11±1.34 and 4.0±1.17 respectively. Overall, during the 3rd trimester four out of 32 women (12.5%) had lower PZ levels, 27 (84.3%) higher and only one (3.2%) had similar PZ levels with the 1st trimester. The observed decline in the PZ levels was between 7 and 130%, whereas the raise in the PZ levels was between 7 and 200% with an average of 60%. All women gave birth to alive and healthy babies however six women (18.8%) gave birth to small for gestation age babies.

Conclusion: Our findings indicate that PZ levels seem to increase during complicated by hypercoagulability pregnancies under LMWH as anticoagulation treatment, similarly to normal pregnancies.

P (Clinical chemistry) -17

The Most Important Metabolic Disorders in Patients Consulting in the Laboratory of the Regional Hospital of Ben Guerdane

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Because of its prevalence and the severity of its complications, diabetes remains an important health and economic problem. Tunisia is among the countries with higher prevalence of diabetes. There are also some other metabolic disorders implicated in several other diseases. This work aims to study the prevalence of metabolic disorders in patients consulting in laboratory of Regional Hospital of Ben Guerdane (Southern Tunisia), the unique laboratory in our region.

The data of 656 patients, distributed over determined period in order to avoid redundancy, were analyzed.

Results showed that 11.2% (11.5% of men and 11% of women) according WHO classification and 22.5% according American Diabetes Association of patients have impaired fasting glucose. This showed considerable prevalence of prediabetes. 31.8% of men and 30.5% of women have fasting glucose greater than 7 mmol/l. The assessment of glycemic control, based on the determination of HbA1c, showed that the proportion of poorly controlled diabetes remains high (58%). Diabetes was also the cause of end stage renal disease in 28% of current dialysis cases. 43.5% of patients have triglycerides level exceeding 1.7 mmol/l and 24.8% have a level above 2.24 mmol/l, that is an independent risk factor for stroke, especially in coronary. 17.6% of women and 18.3% of men have hyperuricemia. In fact, Hyperuricemia causes several diseases such as nephropathy and gout.

This study showed that Hyperglycemia is the most important metabolic disorder, followed by Hypertriglyceridemia, and Hyperuricemia. These data lead us to envisage health education to patients with these metabolic disorders.

P (Hematology) -1

Effects of the Electromagnetic Fields on the Automated Blood Cell Measurements

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Introduction: Nowadays, an excessive use of mobile phones and computers in the laboratory is observed. Consequently, humans and laboratory equipment are exposed continuously in large amounts of electromagnetic radiation. The **aim** of this study is to investigate whether the electromagnetic fields associated with mobile phones and/or portable computers, interfere with blood cell counts of hematology analyzers.

Material and Methods: 80 random blood samples, collected in tubes with K₂EDTA as an anticoagulant, were analyzed on an Aperture Impedance hematology analyzer. The analysis was performed in four ways: A) without the presence of any mobile phone or portable computer in use (control group), B) with mobile phones in use (B1: 1 mobile, B4: 4 mobiles), C) with portable computers (laptops) in use (C1: 1 laptop, C3: 3 laptops) and D) with 4 mobile phones and 3 laptops in use simultaneously. Data of all direct measurements were analyzed using SPSS 16 software for Windows. Normality of continuous data was determined, while paired-samples t-test was used for comparisons in all groups. A two tailed P value <0.05 was considered statistically significant for all comparisons.

Results: From the analysis of our results a statistical significant decrease was observed in neutrophil, erythrocyte and platelet count and an increase in lymphocyte count, MCV and RDW, notably in B4 group. Despite this statistical significance, in clinical practice only the RBC reduction could be taken into account, as the mean difference between A and B4 group was 60.000cells/ μ l. In group D the analyzer gave odd results after 11 measurements and finally stopped working.

Conclusion: The combined and multiple uses of mobile phones and computers affect the function of hematology analyzers leading to false results. Consequently, the use of such electronic devices during their function must be avoided.

P (Hematology) -2

Study of Residual White Blood Cells and Coagulation Factors in Riboflavin Treated Plasma

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Introduction: Due to the remarkable improvements in the blood screening, the blood used in transfusions is safer than ever before. However, there is still the risk of transfusion transmitted infections, because of the "window" period, while emerging pathogens remain an unremitting threat. Furthermore, the presence of residual white blood cells in blood components has the potential to cause immunological implications in patients.

Background: The development of several pathogen reduction technologies can reinforce blood safety, as they render pathogens non-functional, while some of which, can possibly inactivate the remaining white blood cells. Although the efficacy of these methods has been proven, their major drawback is the decrease in the levels of the coagulation factors.

Objective: The current study was an endeavor for the evaluation of riboflavin-based pathogen reduction technology (Mirasol® Pathogen Reduction System, Terumo BCT), in fresh frozen plasma (FFP).

Materials and methods: FFP derived from healthy donors (n=30) was used for the estimation of white blood cells' number via blood analyzer and flow cytometry. Clotting factor VIII and fibrinogen were measured with a clotting-based assay, before and after the riboflavin treatment. Retention of FVIII and fibrinogen was calculated using Riboflavin Control Method (RCM).

Results and discussion: The mean value of the white blood cells' number prior to the treatment was within the requirements¹ (46823.06 cells per FFP bag), a number which is within the specification for the implementation of riboflavin treatment. The mean retention of FVIII and Fibrinogen was 95% (range 71-118%) and 88% (range 62-145%), respectively.

Conclusion: Our results are consistent with the in vitro studies conducted so far. As for the future goals, the ongoing clinical studies are of prime importance, so that more conclusive information of the method's performance can be drawn.

¹ European Committee (Partial Agreement) on Blood Transfusion, Guide to the Preparation, Use and Quality Assurance of Blood Components. 16th ed, ed. S. Keitel. 2010: Council of Europe,.

P (Heamatology) -3

Nucleated Red Blood Cells in Umbilical Cord Blood Units Before and After Volume Reduction Process

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Introduction-Background: Cord blood (CB) contains haematopoietic progenitor cells, including nucleated red blood cells (NRBCs), in small percentages. NRBCs express the CD71 marker on their surface.

Objective: NRBC enumeration in unprocessed and volume-reduced cord blood units (CBU) samples, as well as the study of the potential correlation of NRBCs with several factors.

Material and Methods: 53 CBUs were manually volume-reduced by Rubinstein's double centrifugation method. Cell enumeration was carried out using flow cytometry.

Results and Discussion: The number of NRBCs in unprocessed samples ($141,8 \pm 73,5 \times 10^6$ cells) was higher than in volume-reduced ($69,1 \pm 44,6 \times 10^6$ cells) ($p < 0,001$). There was a positive correlation between NRBCs and gestational weeks ($p < 0,01$). The mean value of NRBCs in CBUs collected after 39-41 gestational weeks was higher than in those collected after 37-38 gestational weeks ($170,7 \pm 75,1 \times 10^6$ cells vs. $101,0 \pm 48,4 \times 10^6$ cells before volume reduction and $80,0 \pm 46,4 \times 10^6$ cells vs. $53,6 \pm 37,8 \times 10^6$ cells after volume reduction). Although there was a correlation between NRBCs and type of delivery, sex and weight of the infant, it was not statistically significant ($p > 0,05$).

Conclusion: The repetition of the study is recommended, using more CBUs and considering more factors which are supposed to increase the NRBC count, such as medication and smoking during pregnancy.

P (Hematology) -4

Cytostatic and Cytotoxic Effects of Selected Inhibitors of c-KIT Receptor on Different AML Cell Lines

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In spite of continuous progress in therapy of acute leukemia, treatment failures still occur frequently. Mutations affecting genes for tyrosine kinases and their signaling pathways result in abnormal proliferation and lead to acute myeloid leukemia (AML). The application of preparations curbing the impact of this disorder might contribute to further improvement of its curability.

The aim of this work was to determine the influence of selected inhibitors of c-KIT receptor tyrosine kinase on growth and survival of AML. We compared the antitumor activities of the multitargeted tyrosine kinase inhibitors imatinib, nilotinib, midostaurin and dasatinib to determine which inhibitor causes the strongest cytostatic and cytotoxic effect on AML cells.

The human AML cell lines HL-60, Kasumi-1, MV-4-11 and THP-1 were cultured in RPMI 1640 containing 10% or 20% fetal bovine serum. Cell proliferation was determined by hemocytometer counts and MTS assay. The investigated tyrosine kinase inhibitors were also examined for their cytotoxic potential and ability to induce tumor cell apoptosis or necrosis. The percentage of apoptotic cells was determined by differential staining with Hoechst No. 33258 and propidium iodide (PI) and FACS analysis using Annexin V/PI staining.

The exposure of acute myeloid leukemia cells to investigated inhibitors at the concentration $\geq 0.01 \mu\text{M}$ resulted in dose-dependent suppression of proliferation compared to the control. Imatinib, nilotinib, midostaurin and dasatinib completely inhibited growth of AML cell lines. Both differential staining and FACS analysis showed independently that investigated inhibitors induce apoptosis of AML cells. The percentage of apoptotic cells was increased in a dose-dependent manner by treatment with inhibitors. Midostaurin and dasatinib are more potent inhibitors of cellular proliferation than imatinib and nilotinib. Moreover, they demonstrate stronger proapoptotic effects. Tyrosine kinase inhibitors such as imatinib, nilotinib, midostaurin and dasatinib represent a promising class of therapeutic agents for the treatment of AML.

P (Microbiology) -1

Detection of *Mycobacterium Tuberculosis* by PCR

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Introduction: Tuberculosis (TB) is the infection caused by *Mycobacterium tuberculosis* and usually affects the lungs but can also occur as extra-pulmonary or disseminated disease. It constitutes a public health problem worldwide with a global mortality of 1.2 to 1.5 million. Early diagnosis of infected patients and application of therapy are the main prevention strategies for its control.

Materials and Methods: In this study, which was conducted in 2 years, 1785 clinical samples were collected from patients with suspected *M. tuberculosis* infection. They were tested by PCR assay (1545 samples were tested by the Cobas Amplicor MTB -TEST and 240 by the Cobas Taqman MTB-TEST, Roche Diagnostics).

Results: From 1785 samples processed, 1139 (63.81%) were sputum and bronchoscopy lavage samples, 318 (17.81%) urine, 199 (11.15%) pleural fluid, 98 (5.49%) gastric fluid, 17 (0.95 %) CSF, 5 lung tissue samples, 7 ascites fluid, 2 pericardial fluid, 5 peritoneal fluid, 1 synovial fluid, 2 pus samples and 1 ear fluid sample. In total, 72 (4.03 %) samples were positive. Out of these, 50 samples were sputum and belonged to patients with pulmonary infection, 18 were urine, 1 pleural fluid, 1 lung tissue sample, 1 gastric fluid and 1 ear fluid. Mixed infections by *M. avium* or *M. intracellulare* were not observed.

Discussion: PCR has an important role in the diagnosis of TB both in pulmonary and extra-pulmonary samples. PCR based assays offer high sensitivity by amplification of small amount of DNA and direct detection of *M. tuberculosis* in a significantly shorter time. Acid-fast stained smears and culture, which are the standard procedures of TB diagnosis, are low in sensitivity and time consuming

P (Microbiology) -2

Differentiation of Clinical Staphylococcal Isolates, Including MRSA, by MALDI-TOF-MS

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Background: Limited success has been reported for matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) differentiation of staphylococci, including MRSA strains, using non- or partially-optimized protocols.

Objective: The objective of the research was to enhance differentiation of clinical staphylococcal isolates through systematic optimisation of MALDI-TOF-MS parameters, with evaluation of the protocol by applying to three sets of isolates.

Material and Methods: Three sets of clinical isolates were used: methicillin sensitive *S. aureus* (MSSA; 36 isolates); methicillin resistant *S. aureus* (MRSA; 30 isolates); other *Staphylococcus* species (NSA; 15 species, 48 isolates). Combinations of chemical pre-treatment of cells on target plates (solvents, reductants, detergents, acids) were introduced to enhance spectral richness. Other parameters for optimisation were: growth medium; matrix chemical; target plate inoculation method; and peak picking/processing criteria.

Results And Discussion: The optimised protocol involved: growth on Muller Hinton agar; single colony transfer to target plate; application of formic acid:isopropanol:H₂O (13:33:50) as chemical pre-treatment; use of α -cyano-4-hydroxycinnamic acid as matrix chemical in ACN:H₂O (2:1) and 2% trifluoroacetic acid; and discrimination by species specific peaks rather than by total peaks. Inclusion of the chemical pre-treatment step, in particular, increased the number of species specific peaks detected over the 2,000-7,000m/z range; typically 34 *S. aureus* specific peaks within a total of 754 detected. MRSA isolates were differentiated from NSA isolates with 100% accuracy, and from other SA with 77% accuracy.

Conclusions: Enhanced differentiation of clinical staphylococcal isolates by MALDI-TOF-MS can be achieved through systematic optimisation of parameters. Application of pre-treatment chemicals to intact cell is highlighted as a key step in the optimisation, although differentiation of MRSA from MSSA isolates was not achieved with 100% accuracy.

P (Microbiology) -3

Diversity and virulence mechanisms in bacterial communities adapted to inert surfaces

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Introduction: We live in a society where the bacterial strains are increasingly adapted to the environment. These are responsible for serious infections, due to a number of virulence mechanisms that have developed over time. Antimicrobial resistance and the ability to form biofilm are two examples of different mechanisms that microorganisms can use to cause disease in humans.

Material and methods: Microorganisms were isolated from samples collected in handrails of escalators of a shopping center and their identification was made using the Api system. Then, the sensitivity test to antibiotics was performed by disc diffusion method (Kirby-Bauer) and the determination of the ability to form biofilm by the staining method with crystal violet.

Results: In this study, 14 different species were identified, highlighting the Staphylococcus species, such as *S. epidermidis* (27%) and *S. hominis*(5%). Following Streptococcus species (*E. faecalis* and *S. agalactiae*), *Shigella* sp., *Citrobacter freundii* and *Acinetobacter calcoaceticus*. We observed the following profiles of antibiotic resistance: in the Streptococcus genus resistance to vancomycin; Staphylococcus genus resistance to oxacillin and trimethoprim; Enterobacteriaceae family resistance for ampicillin, cefotaxime and ceftaxime. Approximately 18% of the microorganisms studied showed ability to form biofilm. Relating more ability to form biofilm and greater resistance, we observed some a tendency.

Discussion/Conclusion: In this study there was a profile of antibiotic susceptibility related on the different strains tested. The resistant phenotype was observed in antibiotics used in the clinic to treat opportunistic infections caused by this type of agents. The concepts of biofilm and antibiotic resistance shown to be very important to survival and adaptation of microorganisms in this type of environments.

P (Microbiology) -4

Microbiological analysis in peripheral venous catheters: microbial diversity and virulence

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Introduction: Bacterial colonization of peripheral venous catheters occurs primarily through the skin of the patient and may lead to infectious complications ranging from infection of the site of insertion to a bacteremia. *Staphylococcus epidermidis* is the most common infectious agent in these cases. Therefore, the bacterial diversity that colonizes peripheral venous catheters was analyzed and identified and their virulence for antibiotic resistance and ability to form biofilms were evaluated.

Material and methods: The study involved microbiological evaluation of 337 peripheral venous catheters in CHUC hospitalized patients, as well as the respective swab of the skin around the catheter insertion site. The identification of microorganisms was made using the semiautomatic Api method. Phenotypic analysis of antibiotic sensitivity was determined using the disk diffusion method and finally it was tested the ability to form biofilm with staining method of crystal violet.

Results: In this study the more abundant bacteria were *Staphylococcus epidermidis* (36%). For this species we found a profile of resistance for the antibiotics ciprofloxacin (58%), gentamicin (33%), oxacillin (27%), ampicillin (9%) and trimethoprim (33%) and a high capacity to form biofilm. Regarding antibiotic resistance and the ability to form biofilm in different species, we observed that bacteria with increased resistance profile have greater tendency to form biofilm.

Discussion/Conclusion: The strain with greater clinical relevance in the present study was *Staphylococcus epidermidis*, which is in agreement with previous studies. This species is commensal of the skin and for this reason it has a greater potential to colonize peripheral venous catheters. This colonization occurs especially when the catheter is inserted. The persistence of these bacteria in these devices is mainly due to the high capacity of these strains in order to form biofilm and consequently greater potential to cause human infections.

P (Microbiology) -5

Screening Acute Myeloid Leukemia Apoptosis-inducing Activity from Forty Cyanobacteria Strains

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Cyanobacterial bioactive compounds can be explored for drug leads. In this study forty cyanobacteria strains isolated from marine and symbiotic lichen habitats were screened for apoptosis-inducing activity against acute myeloid leukemia cells. More than half of the strains showed cytotoxicity against rat acute myeloid leukemia. The toxicity was not due to the known cyanobacterial hepatotoxins (microcystin and nodularin) or known metabolites with anti-leukemic activity, such as adenosine and its analogs as earlier detected by cell experiment and LC-MS with nodularin and adenosine deaminase. The toxicity test of HEK293T cells indicated that the potent extracts induced apoptosis selectively in leukemia cells. Six of the forty extracts exhibited significant anti-AML activities. Among them, extract L26O presented the best anti-leukemia activity. Extracts L26O and L30O were able to counteracted the chemotherapy resistance resulted from the oncogenic protein Bcl-2. Extract L1O cooperated with the tumor suppressor protein P53 to induce increased apoptosis of human leukemia cells. In conclusion, cyanobacteria are a prolific resource for anti-leukemia compounds for pharmaceutical applications.

Keywords: cyanobacteria, acute myeloid leukemia, microcystin, p53, Bcl-2, apoptosis.

P (Microbiology) -6

Functional Polymorphisms in the TLR7 and TLR8 Genes Contribute the Susceptibility to Mycobacterium tuberculosis Infection

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Tuberculosis (TB) recently has re-emerged as a major public health threat worldwide and Mycobacteria tuberculosis is a highly successful pathogen evolved remarkable strategies to establish persist infection. There is strong evidence that host genetic factors influence individual susceptibility to TB. A large number of studies have investigated whether polymorphisms in the Toll-like receptor (TLR) genes are implicated in susceptibility to TB. We evaluated the associations between TLR7 and TLR8 gene SNPs and susceptibility to TB in Chinese. Our results demonstrated that TLR8-129C allele was present at higher frequency in males TB infected group as compared to the healthy control group (24.1% vs. 6.8%, $p=0.001$). Based on haplotype analysis, the frequency of wild TLR7 IVS2-151A/TLR8 -129G was significantly lower in TB infection patients than control subjects (73.0% versus 83.2%; $p0.001$). The haplotype TLR7 IVS2-151A/TLR8 -129C increased the risk for TB infection compared to wild type TLR7 IVS2-151A/TLR8 -129G, with OR= 3.87 (95% CI = 1.78 to 8.43; $p0.001$). Furthermore, the functional effects of these polymorphisms on the defense against M. tuberculosis by ex vivo phagocytosis assay. Our data revealed the monocyte of individuals with the haplotypes TLR7 IVS2-151A/TLR8 -1129C have significantly higher phagocytosis ability than those with wild type TLR7 IVS2-151A/TLR8 -129G (64.0±11.8% versus 32.1±14.0%; $p=0.041$). Mycobacteria within monocyte can escape the immune destruction; therefore, individuals with the haplotypes AC might less susceptible to TB infection due to less phagocytosis ability. In conclusion, these findings provide an evidence of association between TLR7 and TLR8 gene polymorphism with susceptibility to TB infection correlated with the monocyte TB phagocytosis.

P (Microbiology) -7

The New Era of Antiviral Therapy Against Hepatitis C Virus

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Introduction: More than two decades of intense research has provided a detailed understanding of hepatitis C virus (HCV), which chronically infects 2% of the world's population. Hepatitis, which can be also caused by HCV, is a disease caused by inflammation of the liver. Hepatitis C virus and especially therapy of chronic patients preoccupies science since there are more than 170 million the HCV carriers as it has been estimated and moreover there is no preventive or therapeutic vaccine.

Background: This effort has paved the way for the development of antiviral compounds to spare patients from life-threatening liver disease. The Standard of Care therapy, (SoC), attributed to combat HCV, included the administration of pegylated interferon and ribavirin in various regimens. An exciting new era in HCV therapy dawned with the recent approval of two viral protease inhibitors, used in combination with pegylated interferon- α and ribavirin (SoC); however, this is just the beginning.

Objective and methods: The purpose of the present study was to literature review the up to now bibliography on newly developed and those are under development HCV-specific drugs. An extensive search was conducted in pubmed and google.scholar to retrieve and study the relative bibliography.

Results: Multiple classes of antivirals with distinct targets promise highly efficient combinations, and interferon-free regimens with short treatment duration and fewer side effects are the future of HCV therapy. Until today, only two protease inhibitors, telaprevir and boceprevir, have been approved and marketed both in America and Europe, and are used in various therapeutic regimens in proportion to the viral genome and the response to the SoC therapy. Ongoing and future trials will determine the best antiviral combinations and whether the current seemingly rich pipeline is sufficient for successful treatment of all patients in the face of major challenges, such as HCV diversity, viral resistance, the influence of host genetics, advanced liver disease and other co-morbidities.

P (Cytology) -1

Differences in Prevalence of Precancerous Lesions in Three Different Populations Participating in the National Cervical Cancer Screening Program

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Introduction: Cervical cancer is the second most common cancer in women worldwide and seems to be influenced by ethnic factors, socio-economic status and access to screening programs.

Objective: The aim of the study was to compare the prevalence of cancer, precancerous lesions and infections detected in pap-smears from three different health care providers, reflecting populations differing in socio-economic status and nationality composition.

Materials And Methods: This is a cross-sectional study of 1500 pap-smear tests (Bethesda classification) obtained from three sources: a central hospital in Athens serving a middle socio-economic status group (group A, N=500), a community infirmary in Athens serving a predominantly vulnerable socio-economic population (group B, N=500) and provincial community infirmaries in Crete, serving a rural population with limited access to health care centres (group C, N=500). X^2 test was used for statistical evaluation.

Results And Discussion: The results for groups A, B and C respectively were: 5,8%, 1,4% and 1,4% unsatisfactory, 62,8%, 55% and 57,4% negative, 24,6%, 39,4% and 36,4% inflammatory, 3,6%, 1,4% and 1,4% ASCUS, 3%, 2,8% and 1,4% intraepithelial lesions, and 0,2% squamous cell carcinoma in the group A. Taking under consideration nationality data, there were 3,6% and 2,9% unsatisfactory, 59,5% and 50% negative, 32,3% and 42,5% inflammatory, 2,3% and 0,6% ASCUS, 2,3% and 4% intraepithelial lesions for women with or without Hellenic nationality respectively.

Conclusion: Group A presents higher prevalence of ASCUS, precancerous lesions and cancer and lower prevalence of inflammation than B and C (p-value 0,0001). There is also a mild preponderance of precancerous lesions and inflammation detection in immigrants (p-value=0,022). These results may lead to the conclusion that women that belong to the middle socio-economic status, being better informed about the importance of pap-test, take regular asymptomatic screening, while immigrants and low socio-economic status women seek medical attention only once symptomatic.

P (Cytology) -2

A comparative study of Liquid-based and Conventional Cytology in cervical smears of young women.

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Introduction: Cervical cancer is the most common female cancer in the developing world. Its incidence is significantly lower in USA, Europe and other developed countries because of the organized screening programmes which identify women at high risk of cervical cancer.

Background: Cervical cytology screening since the mid-20th century has remained the most successful cancer screening programme to date. It's based on Papanicolaou (Pap) smears (Conventional Cytology). But the Pap test is not flawless because of an interpretative sample, bad preparation and staining errors which can reduce the diagnostic accuracy of the method. In many countries conventional cytology (Pap smears) has given way to Liquid – based Cytology (LBC), such as Thin Prep and SurePath, approved by the U.S. [Food and Drug Administration](#) (FDA).

Objective: This study compares conventional cytology with liquid-based (LBC) for primary cervical screening in a randomized group of young women aged 18-25 years.

Material and Methods: 45 women aged 18-25 were screened with conventional and liquid – based cytology (Sure Path). A sample of cervical cells was taken using cervix brush for the two cytological methods. The second sample is vortexed, strained, layered onto a density gradient, and centrifuged. The cells form a circle 12.5 mm in diameter. Smears were classified according to the Bethesda 2006 Reporting System.

Results and Discussion: Our results have shown distinct advantage of LBC over conventional cytology. In LBC a well preserved, approximately monolayer of cells is prepared with fewer unsatisfactory results that are primarily due to obscuration by inflammation, blood and mucus or due to inappropriate spread or fixation of cells. LBC preparations can also be interpreted more quickly while residual material can be easily used for ancillary immunocytochemical and molecular testing.

Conclusions: We concluded that unsatisfactory smears or limited smears by the presence of inflammation and red blood cells are statistically less important with LBC than with the conventional method. Note that the absence of cellular material due to a sampling of bad quality remains as frequent in LBC as in conventional smear.

P (Cytology) -3

Benefit from the use of specific ancillary techniques in effusion cytology

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Occasional effusion cytology cases needs the use of ancillary studies for an accurate diagnosis. The demand of specific ancillary techniques is based on the cytomorphologic findings.

- Immunocytochemical studies when used as a panel are the most helpful of the ancillary techniques in the workup of a difficult effusion cytology case.
- Routine histochemical stains for mucin should also be employed due to their low cost and simplicity.
- Immunophenotyping by flow cytometry is useful in the workup of problematic lymphoid proliferations
- Electron microscopy (EM) examination is especially helpful in the differentiation of metastatic carcinoma from mesothelioma, but lacks specificity due to overlapping ultrastructural features, and is both costly and labor intensive

P (Cytology) -4

Target preparation of thin-prep PAP tests for molecular analysis

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Purpose: The purpose of this presentation is to reduce the percentage of 'unsatisfactory' Thin-Prep Pap Test samples for further molecular analysis.

Material and methods: The material corresponds to Thin-Prep Pap Test samples, which macroscopically were characterized 'unsatisfactory'. The process which followed was the following:

* Samples with mucous >[Filtration & Dilution.

* Bloody samples > Lysis of RBCs using Cytolyt solution

* Sparse cellularity samples > Centrifugation at 1500 rpm for 10 min & dilution the precipitate in 5ml Preserv Cytolyt solution.

HPV typing was done using microarray technique (Clinical Arrays Human Papillomavirus, CLART[®], GEMONICA, Spain).

Results: After optimized preparation of the samples which were initially unsuitable, HPV typing at 67.2% of them was possible.

Conclusion: Targeted preparation of Thin-Prep Pap Tests increases their suitability to further molecular analysis, as it significantly reduces the percentage of samples that need to be reanalyzed.

P (Cytology) -5

Usefulness of cell block preparations from liquid-based cytologic specimens

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Aim: In this study, we aimed to use cell remnants from Liquid Based Cytologic (LBC) specimens for preparation of cell blocks for immunocytochemical diagnosis of malignancy.

MATERIAL AND METHODS: The Cellient™ Automated Cell Block System was used which rapidly creates paraffin embedded cell block by means of a controlled vacuum to deposit a layer of cells on a filter and infiltrate those cells with reagents and paraffin. Cell blocks made were immunocytochemically stained for several biomarkers including certain tumor markers, proliferative antigens (PCNA, Ki67) and hormone receptors for accurate diagnosis of malignancies in different samples from needle aspirates from breast and thyroid tumors, and liquid samples (ascites, pleural effusion, and urine)..

Results: The findings from the cell blocks stained with these biomarkers combined with those from Pap stain led to easily diagnosis of the presence or absence of malignancies.

Conclusion: Our findings suggest the utility of LBC and cell blocks from cell remnants in cytologic diagnosis in certain specimens.

P (Cytology) -6

HPV vaccination coverage among female students aged 18-25 years.

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Introduction: Cervical cancer constitutes a serious public health problem in Greece as well as worldwide. Its main risk factor is Human Papilloma Virus (HPV) infection and factors associated with sexual behavior. The most important part of cervical cancer prevention is immunotherapy. Two vaccines, Ardabil and Cervarix, help to develop the appropriate immune response for the prevention of an HPV infection with high risk oncogenic HPV types 16, 18 the major cause of cervical cancer.

Background: Routine immunization of adolescent girls aged 11-18 years against HPV was recommended in Greece in 2007 in a routine vaccination schedule by the Greek National Immunization Program (NIP).

Objective: The aim of this study is to help with the gathering country specific data for the HPV vaccination coverage in Greece with regard on age of sexual debut in young female aged 18-25 years.

Material and Methods: Overall 650 female students ranged in age from 18-25 participated in this study. Data were collected using an anonymous, self-administered questionnaire designed to gather data about HPV vaccination [if they have been vaccinated, the age of vaccination, the type of vaccine (Gardasil, Cervarix), the number of doses they have already done, age at first intercourse].

Results and Discussion: Only 18% of the participants were vaccinated at the age ranging from 16 to 21 (meanage 16.6). 84% of them were vaccinated with Gardasil and 16% with Cervarix. 96% of the vaccinated students reported receipt of a complete 3-dose course of HPV vaccines, while the remaining 4% received only 1 or 2 doses. Furthermore, 79% of the participants have started their sexual activity between 16 and 19 (meanage: 17,4).

Conclusions: HPV vaccination coverage in Greece is very low. Our results indicate that there is an urgent need for the implementation of the coordinated adolescent vaccination programme to facilitate access to vaccination. It's a crucial point; otherwise the HPV vaccination effort will fall short of reaching its maximum public health benefit.

P (Cytology) -7

Inflammatory Conventional Pap smears. Cytomorphological diagnosis of the infectious agents.

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Introduction: Pap smear has been in use for more than half a century as a primary screening test for precancerous and invasive cervical lesions. Cervicovaginal infections are common especially in young and sexually active women. Conventional Pap smear can also be extremely useful in the cytomorphological diagnosis of some pathogenic microorganisms in the cases of cervicovaginitis.

Background: Cervicovaginitis cannot be adequately diagnosed solely on the basis of symptoms or physical examination. Conventional Pap test can help in determining the causative agent of the infection.

Objective: The aim of this study was to determine the role of Pap smears in the diagnosis of lower genital tract infections in young women, and specifically to identify specific pathogenic microorganisms in symptomatic and asymptomatic cases and assess the association between inflammation on Pap smears with the presence of cervical/vaginal pathogens.

Material and Methods: 186 conventional cervicovaginal smears were taken from young women aged between 18- 28 years. Cases without precancerous findings only were included. 88(47.3%) of them complained about vaginal discharge and occasionally about purities vulvae. Six types of pathogenic agents were examined: Candida species, Chlamydia trachoma is, Gardenella Vaginalis, Trichomonas Vaginalis, Actinomyces, Leptotrix Vaginalis. Reasons for unsatisfactory results (obscuration by blood or inflammatory cells) were also examined.

Results and Discussion: The overall unsatisfactory smears were 4.9%. Evidence of inflammation has been found in 37% of the microscopically examined cases. In most of the inflammatory smears (34%) clue cells indicating Gardenella infection have been recognized. 15% of the participants were positive for fungal infection, while 9% for Trichomonas infection.

Conclusions: Conventional Pap smear is well suited for diagnosing cervicovaginal infections. Gardenella Vaginalis is the major problem in the women examined. A repeat Pap smear in 4 or 6 months time is recommended after treatment. If the inflammatory changes still persist the woman must be submitted to further investigation.

P (Molecular biology) -1

Challenges posed by Potter syndrome/sequence genetics: Confirmed etiology vs. Probable etiology

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Introduction: The ARPKD has a familial recurrence rate of 25%, its identification permits family planning genetic counseling. The pre-analytical considerations are essential to ensure certainty diagnosis.

Background: The congenital bilateral nephropathies constitute a highly heterogenous group of diversely caused conditions with an equally varied clinical presentation. Making the study of stillborns of paramount importance as it sheds light on etiology, essential for future genetic counseling and prevention.

Whenever a Potter sequence (i.e. peculiar facies, clubbed feet, pulmonary hypoplasia) is identified in an US, in the context of probable renal-origin oligohydramnios, then a voluntary abortion might be indicated.

Objectives: Ensure optimal sample recollection that affords the best possible genetic-assay

Materials and Methods: A protocol was drawn and communicated to healthcare providers involve in this sub-set of patients management, stating:

According to patient recruitment they can be grouped in two categories subject to different testing
Group liable to confirmed etiology: given positive pathology confirmation, PKHD1 indirect testing by linkage analysis can be undertaken, this will invariable yield conclusive results, all this in an economically and easily performed fashion.

Group subjected to probable-etiology diagnosis: larger group due to US screening and interrupted gestation, where the PKHD1 sequencing is available with a detection rate of 80-85%, this study on the other hand is more complex, more costly and requires an adequate not paraffin-wax imbedded sample.

Results and Discussion: Our service is notified prior to programming an elective abortion, in order to obtain a glut or thigh specimen suspended in saline solution before sending the fetus for pathologic assessment. Once pathology results are out the adequate study will be chosen, or further studies undertaken and familial genetic counseling will be offered.

Conclusion: Our daily practice shows improved results as far as the quality of the information provided to the patient and other attending physicians goes.

P (Molecular biology) -2

Genetic counseling in familial cancer cases of compounded maternal and paternal risk. A propos of a case

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Introduction: The availability of molecular diagnosis for risk-modifying mutations created new patient-management paradigms, where precise information supplied by healthcare providers to patients as well as family members is achieved through genetic counseling.

Background: Biparental risk factors for single-gene conditions are not uncommon. Workup selection and providing accurate information to the family might prove challenging.

Objectives: Describe the molecular diagnosis and genetic counseling for biparental breast and ovarian cancer risk-factors families.

Materials, Methods: 33yo female patient with breast cancer (BC) with c.221AG p.Gln74Arg (originary of NW-Spain) identified as paternal-line mutation

Pedigree:

Paternal:

- father skin cancer
- aunt deceased 62yo ovarian cancer
- aunt deceased 52yo BC
- whose daughter BRCA1
- aunt deceased 52yo BC
- whose daughter deceased 57yo BC
- cousin deceased 62yo colon cancer
- whose daughter BC
- whose daughter BC

Maternal:

- Grandmother deceased 63yo colon cancer
- uncle deceased 76yo pancreas cancer
- whose 2 daughters BC
- aunt deceased 35yo BC
- aunt deceased 64yo colon cancer
- aunt BC

Results-Discussion: Following c.221AG p.Gln74Arg testing, family was informed of an independent maternal-line risk, rendering siblings tests non-informative.

Unaffected individuals would be negative for byparental mutations and subject to the same risk as general population, it's enormously important that this be made clear to the family, since underestimating the risk could lead to delayed diagnosis and decreased survival

A proband from the maternal line has been selected to enable the independent assessment of the affected genes. It would be reasonable to expect the same mutation, considering common geographic origin.

Conclusions: Two deleterious mutations in BRCA have not been described yet; considering that a negative result for one mutation does not rule out the possibility of the other, thus not modifying patient management, thus partial testing in the siblings would prove ineffective.

P (Molecular biology) -3

Partial Hidatidiform Mole a Propos of a Case Cytogenetic Diagnosis During Foetal Gestation

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Introduction: Gestational-trophoblastic diseases are conditions originating in the placenta including partial and complete moles

Background: Partial hidatidiform-mole characteristically presents triploid chromosomal complement either resulting from paternal duplication or dispermy, with a typical 69XXY karyotype, affecting one in 22000-100000. With a risk of malignant progression of 10% and 40% chance of uncomplicated life-birth.

Objective: Describe a gestation complicated by a hidatidiform mole; and its cytogenetic testing. Underline the special considerations for such testing.

Material, Methods: 33yo female, with septate uterus, referred to high-risk-consultation after an ultrasound revealing single fetus without detectable alterations and 2/3 hydropic placental degeneration. 10th week serum β hCG 92000mIU/ml; suggestive of partial-mole.

Bilateral placental biopsy is programmed, electing samples from both the region showing degeneration and the "normal" region, to permit comparison.

Results and Discussion: The abnormal specimen shows globular and unstructured villi, QF-PCR (Ch13, 18, 21, X/Y) is non-conclusive, raising the possibility of multiple cell-lines in the sample prompting karyotype study. Whereas the "normal" specimen indicates female euploid fetus (46XX).

Subsequent karyotype for abnormal specimen shows 46XX, but viable metaphases are identified in only 1-in-3 cell-cultures, raising the possibility cell-lines admixture and/or partial lost.

A later US (14+2wks) is positive for occlusive placenta praevia, showing 2/3 normal placental tissue and 1/3 hydropic degeneration; whilst β hCG descended (i.e. 78699mUI/ml 12th wk and 42521mUI/ml 14th wk).

Conclusion: The fact that there is an inversion of normal to hydropic placental tissue in the follow up US might be indicative of non progression of the partial mole or in this case in point might call into question its classification as such.

Biopsy specimens for mole studies requires that both samples the one suggestive of hydropic degeneration and the normal gestational tissue are well isolated so as to yield independent cytogenetic results that might allow for comparison between the sample set.

P (Molecular biology) -4

Optimized Overhang Expansion PCR protocol for higher DNA yield

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Introduction: Overhang-Expansion-PCR(OE-PCR) enables both site-directed-mutagenesis and deletions to be introduced in a single step, predicating on two sequential PCR reactions, the primary uses the Wt-strand as template and two sets of primers were one of the sets forwards overlaps with the other's backward coded-sequence; including the desired mutation. The secondary PCR uses the amplicons for the primary as templates and the flanking primers from the primary, yielding the desired mutation containing amplicon.

Background: OE-PCR requires the use of high-efficiency(HF) polymerases that tends to result in lower yields, and requires a reduced number of cycles, further contributing to low-yield as this strategy's drawback.

Objective: Design a protocol to increase OE-PCR yield without compromising efficiency.

Material and Methods: Using Q5HF DNA-polymerase and Phusion-polymerase(NEB-UK), a series of redundant primary OE-PCR reactions were run on a 50uL final volume, then the desired length fragments were purified in a commercial silica membrane using increasing concentration of saline media, and finally eluting with double distilled water, then the elution from one duplicate was loaded with an unpurified duplicate into a fresh column adjusting volumes of saline buffers accordingly but eluting with the same volume of ddW.

Results Discussion: The sample obtained from the sequential duplicated elutions was measured for DNA content by OD and then sequenced to verify fidelity.

Each duplicate reaction eluted increased the final DNA content short of 1-fold the regular yield obtained by single reaction, this strategy proves both simple and relatively inexpensive ensures higher yields of DNA without resorting to lower fidelity polymerases or the addition of other substances to the reactions such as DMSO that might compromise efficiency to increase yield.

Conclusion: Duplicate reactions and sequential purifications steps bypass that low yield offered by regular OE-PCR strategies, and might be applied to other strategies that use high fidelity polymerases

P (Molecular biology) -5

The Distribution of Apolipoprotein E Gene Polymorphism in a Polish Rural Population

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Life-history theory predicts the existence of trade-offs between reproduction and lifespan. Some of these trade-offs may have a genetic background. Human apolipoprotein E (ApoE) plays an important role in several metabolic processes, such as lipid transport and neuronal repair/protection. It is also involved in pathologies related to advanced age, such as cardiovascular and Alzheimer's disease. ApoE also plays an important role in the regulation of steroid hormone action and thus may influence reproduction. The purpose of the study was to examine the distribution of APOE gene polymorphisms in the Polish rural population. The information concerning the subjects involved in the research was collected in the "Mogielica Human Ecology Study Site" in the years 2011-2012. Molecular study included 80 women after 45 years of age. Genotypes were determined in DNA from peripheral blood lymphocytes. APOE gene polymorphism was analysed performed using PCR-RFLP. The allele frequencies in this population were: ApoE3: 77,5% (62/80); ApoE2: 11,25% (9/80); ApoE4: 11,25% (9/80). It is well established that carriers of the APOE3 allele have a higher risk of health problems than APOE2 or APOE4 carriers. Surprisingly, the APOE3 allele is the most frequent in all populations investigated up to now. This high frequency of the health-deleterious APOE*3 allele may be explained by the fact that, as indicated by some studies, APOE*3 carriers may have better health in young age and higher fertility.

P (Molecular biology) -6

Cytogenetic profile OF505 patients with chronic lymphocytic leukemia in Greece

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Background: Conventional and Molecular Cytogenetics is an important prognostic and diagnostic tool in chronic lymphocytic leukemia (CLL).

Objective: We present a systematic cytogenetic study of 505 B-CLL cases to define the chromosomal abnormalities and their frequencies in Greek population, using conventional and molecular cytogenetic methods.

Materials and Methods: Karyotypic analysis was performed on unstimulated and stimulated with B cell mitogens bone marrow cells from 505 patients, aged 16-88 years. Fluorescence in situ hybridization (FISH) studies were carried out in 87 patients using the CLL set probes LSI p53/LSI ATM και LSI D13S319/LSI 13q34/CEP12 Multi-Color Probe Sets.

Results: The sex ratio (males/females) was 2M/1F. The median age of the patients was 64.75 years. Karyotypic analysis was successful in 93.7%. Normal karyotypes were found in 58% and abnormal in 42% of patients. Among the abnormal karyotypes 27.3% exhibited complex karyotypes and 51.5% only one aberration. The most frequent chromosome aberrations in abnormal karyotypes were: +12 (29.3%), -Y (15.7%), abnormal (abn) 17 (13.1%), abn14q (10.6%), del(13q) (9.6%), del(6q) (6.6%), -X (5.6%), abn18 (5.6%) and del(11q) (5%). The most common abnormalities found in karyotypes as sole aberrations were +12 (27.7%), -Y (16.8%), del(13q) (5.9%), add(14q) (4.9%) and del(11q) (4%). FISH analysis showed del(13)(q14.3) in 73.3%, del(13)(q34.3) in 5.0%, del(17)(p13.1)/p53 in 16.6%, +12 in 21.6% and del(11)(q22.3)/ATM in 13.3%.

Conclusions: Karyotypic analysis revealed a variety of chromosome aberrations. Trisomy 12 was the most common abnormality in karyotypes while the submicroscopic del(13)(q14.3) was the most common aberration detected by FISH. The differences in the frequency of chromosomal aberrations between conventional and molecular cytogenetics can be attributed to the ability of FISH to detect submicroscopic aberrations. However, karyotype can reveal all the chromosomal aberrations through the genome. Therefore, the above indicate the necessity for the parallel application of karyotype and FISH in CLL investigation.

P (Immunology) -1

Evaluation of Sequence-Based Typing in High Resolution HLA Typing of Volunteer Hematopoietic Stem Cells Donors

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Introduction and Background: The main hematopoietic organ is bone marrow that produces the Hematopoietic Stem Cells (HSC). To achieve a successful transplant, there should be compatibility between the patient and the donor, which depends on the similarity of the molecules of the Human Leukocyte Antigens complex (HLA), found on most cells' surface.

Objective: The evaluation of the Sequence-Based Typing (SBT) method as one of the most advanced methods that can give immediate high resolution results, since it is able to detect differences at the level of nucleotide.

Material and Method: In order to determine the possible compatibility, before each transplant, a typing of the HLA alleles is performed. In current study SBT method was implemented in 100 whole blood samples received from possible volunteer HSC donors. Due to ambiguous results, a second sequencing, using Heterozygous Ambiguity Resolution Primers (HARPs), was needed to achieve a clearer typing result.

Results and Discussion: During first sequencing, only 9 samples did not have ambiguities. During second sequencing with HARPs, 63.7% of the 91 ambiguous samples had ambiguities in locus A, 60.4% in locus B, 42.9% in locus DRB1, 24.2% in both A and B loci, 12.1% in both A and DRB1 loci, 8.8% in both B and DRB1 loci and 11% had ambiguities in all loci. After the second sequencing, the percentage of the resolved samples was 81.3% (74 samples), while there was an 18.7% (17 samples) of which was not possible to resolve the ambiguities even with using HARPs.

Conclusion: SBT consist a very reliable and high resolution method for HLA typing that can resolve a great percentage of ambiguities using HARPs. In conclusion, it would be significant to expand the study to a larger number of samples using newer HARPs and also with the complementary application of other molecular typing methods like PCR-SSP and PCR-SSOP.

P (Immunology) -2

Expression of MMP-2, MMP-9 and TIMP-2, TIMP-1 in Multiple Sclerosis

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Introduction: Multiple sclerosis (MS) is an inflammatory, autoimmune central nervous system (CNS) disease of unknown etiology, with demyelination of axons causing a context of several physiological and psychomotor disabilities. In MS, matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9 might play an important role in effectors functions such as disruption of the blood-brain barrier (BBB), brain parenchyma invasion by immune cells and demyelination. Therefore is important evaluate serum levels of MMPs and their inhibitors in patients with MS contributing in this way in the development of new strategies for prognosis and therapy of this disease.

Objective: Evaluate the serum levels of MMPs and TIMPs in patients with relapsing-remitting (RRMS), and relate them to the severity of MS.

Material and methods: The study was conducted in 24 subjects, divided into two groups, 12 patients with MS (RRMS) and 12 controls. The semi-quantification of serum levels of MMPs and TIMPs was performed by slot-blot, and the optical density analysis of the bands was performed using the GELDOCTM XR + (Bio-Rad Hercules, USA) image acquisition system and software ImageLab[®] Version 3.0 (Bio Rad, Hercules, USA). Statistical analysis was performed with GraphPad Prism version 5.0. We used Mann-Whitney U and Student t tests.

Results and discussion: There were always recorded higher values in patients compared to control group in all parameters, with the exception of MMP-2. However the differences between groups were not statistically significant. Our results agree with those of other authors.

Conclusion: In our study we point out the importance of further research in order to evaluate and clarify the performance of MMP-9, MMP-2 and respective TIMPs as biomarkers in monitoring, prognosis and new therapies for multiple sclerosis.

P (Immunology) -3

Correlation of methods for detecting autoantibodies in patients of general hospital

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Introduction: The purpose of the immune system is the destruction of the foreign invaders (i.e. microbes) and the protection of the body. In pathological situations however, the immune system might act against his own body and not against foreign ones. Due to that, autoimmune diseases do appear (systemic lupus erythematosus, rheumatoid arthritis, Sjogren syndrome vasculitis). Genetic, hormonal and environmental factors and stressful stimuli seem to be accountable for the these diseases whose the frequency has increased during last decades.

Purpose: Our aim was the correlation of values and detecting methods of autoantibodies in patients of general hospital.

Material-Methods: During 2012, 563 patient blood samples have been studied which were received from the Immunology laboratory of G. GENNIMATAS General Hospital of Athens. In those patients antinuclear antibodies have been traced, antibodies against double helix DNA and antibodies against extracted antigen core. In parallel during the same period, the blood sample of 638 patients have been studied, which was received from the Immunology laboratory; in these samples antibodies against cytoplasmic neutrophils have been traced. The tracing of ANA has been performed with the indirect immunofluorescence method in substrate Hep-2 cells (Inova, USA), the anti-dsDNA with immunoassay method ELISA (Eurodiagnostica, Sweden) and the ENAs (Ro,La,Sm,URNP) with the method of Immunoblotting (Innolia, Innogenetics, Belgium). The tracing of ANCA has been done with the IFA (Inova, USA) and ELISA (MPO, PR3 BL Diagnostica, Germany) method. Results have been statistically assessed using MS Excel.

Results: Out of 563 samples that were checked for ANA, 110 (19,5%) were positive (ANA fluorescent), 148 (26,3%) were slightly positive and 305 (54,2%) were negative. 81,4% (from 110) of the positive samples ANA were of women and 18,6% (out of 110) were of men. With regards to the kind of fluorescent of the positive samples, 83% (out of 110) presented thin fluorescent tics, 9% (out of 110) nucleolar fluorescent, 5,5% (out of 110) cytoplasmic fluorescent and 2,5% (out of 110) presented rough fluorescent. Out of the total 110 positive ANA samples that have been checked concerning DNA, anti-ENAs (SSA/Ro, SSB/La, Sm and URNP) have been traced: 15 samples (45%) with positive anti-dsDNA, 11 (32%) with positive SSA/Ro, 2 (6%) with positive SSB/La, 2 (6%) with positive Sm and 4 (11%) with positive URNP.

Out of 638 samples that have been checked for ANCA with IFA, 23 samples (3,6%) presented perinuclear fluorescent (p-ANCA), 12 (1,8%) cytoplasmic fluorescent (c-ANCA), 6 (0,9%) informal cytoplasmic fluorescent and the rest 597 samples (93,7%) were negative. Out of 12 samples which have been found with ANCA through IFA, 9 (75%) had anti-PR3 with ELISA. Also 8/12 (66%) c-ANCA positive samples were concerning men whereas 4/12 (34%) women. Out of the 23 samples in which p-ANCA with IFA have been traced, 16 (70%) had anti-MPO with ELISA. Also, 18/23 (78%) p-ANCA positive sample were concerning woman whereas 5/23 (22%) men. Simultaneous tracing of c-ANCA and p-ANCA had not been performed in any sample.

Conclusions: Antinuclear antibodies and c-ANCA were found in greater numbers in women, while p-ANCA in men. Indirect immunofluorescence for detecting ANA, in initial dilution of 1/160 is useful for finding the positive samples (ANA > 1/160). Further determination of DNA and ENAs should be done only in positive samples of ANA. Additionally, the indirect immunofluorescence is more sensitive in the detection of ANCA comparing to ELISA, which confirms its result.

P (Laboratory management and Informatics) -1

Reduction of Human Negligence of Laboratory Specimen Errors by Implementation of a Barcode System

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In these years, "Patient safety" is an important issue. In the record of Taiwan Patient Safety Reporting System at 2012, the Laboratory specimen errors happened around 8.1% of all reported cases. Take a hospital at southern Taiwan as example, the Laboratory specimen errors happened 44.7% in their safety reports. Amount those cases, there are 96.7% happened in the specimen collection stage. Unlabeled, mislabeled or wrong patient was the mostly happened situation. Human negligence was the main factor. Therefore, in the inspection and laboratory specimen process implement with identification system is an improvement for reduction the laboratory specimen errors. We implemented a barcode system in the emergency department (ED). Patient was wearing a wristband which contains a barcode linked to the patient's identity. Provider laboratory orders through physician order entry create a message sent to the hospital laboratory information system, containing the patient's name, medical record number, requested laboratory test and specimen, and test code. Before each step for the specimen collection, the staff had to scan the barcode to identify the patient. There are 157,143 specimens were collected from ED in 2012, 389 specimens (0.24%) were recorded as errors for human negligence. In June 2013, barcode system was implemented. 28,674 specimens were collected in next two months, 3 specimens (0.01%) were recorded as errors for human negligence. The safety report of laboratory specimen errors in the ED was reduced from 29.2% to 6.8%. The purpose of laboratory specimen is to provide clinicians diagnosis, therefore the correctly specimen collection is quite important. The barcode technology was primarily used for patient identification, patient care and time management. Application this barcode system for patient identification reduced the laboratory specimen errors and avoided the human negligence. Increasing the medical quality and ensure the patient safety. Furthermore, we suggest that this system should be applied to all medical treatments to identify patient and increase the patient safety.

P (Laboratory management and Informatics) -2

New software for storing and retrieving chemicals reagents information

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Introduction: The recording of the reagents of an educational institution that has educational and research laboratories in different locations and even in different buildings, is critical for the smooth operation of each faculty including laboratory courses and research experiments and its administration, at the lowest cost and in the most productive manner.

Purpose: Our purpose was to create original software which contains all useful information about the chemical reagents which are used in the Medical Laboratory Department. The data include all technical information, instructions and safety information (MSDS document).

Material and Method: The program runs on Windows Operating System. Has been developed in VBA programming language using Microsoft Access DB tools. All information about the chemical reagents that can be detected in various areas of the Medical Laboratory Department of TEI Athens has been entered. Some information i.e. about kits or other materials which are considered reagents like nutrients has been excluded. The software runs in a network environment (mainly in a Drop box platform) and there is the possibility to access and operate from all laboratories of the department.

Results: The data input started just after the development of the software. We input data related to almost 300 different chemical reagents in solid or liquid form. For each chemical product the following information was recorded: Greek and international Name, Molecular Weight, Molecular Formula, Toxicity / Hazard risks, Type (for example salt, base, solvent), Suitability (purity) for chemical analysis, CAS Number, On-line link to MSDS document, Manufacturing company, Location (cabinet / rack) in the laboratory. On top of that, there was an extensive study of bibliography concerning the use of each reagent in experiments either within our department or outside of it.

Conclusions: As of now, this software has already proved to be extremely helpful in the educational and research work of our department. It enables all interested parties not only to locate a chemical reagent in the fastest way, but also to find useful information such as the MW, to assess the risks of using it and even the experiments in which it might be used.

P (Ethics, the future of the profession)

Strategy of Solution for the Transfer to Practical Skills

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The acquisition of occupationally specific core competence in the course of becoming a biomedical scientist in the 21st century will be influenced by current Internet technologies. This work shows to what extent Internet tools influence the core competence, that have to be acquired during the education as a biomedical scientist in the course of biomedical science at the University of Applied Science in Carinthia. The studied core competence is the strategy of solution of conversion into practical skills. In the course of the empiric study a qualitative experiment has been conducted. The possibility of Internet access has been manipulated to study its influence on the solving strategy of each participant. Almost all accesses of the Internet were intended to translate English verbs into German ones. The students indicated that one needs to have a profound theoretical and practical basic knowledge to make purposeful use of Internet tools in this context. Summing up the application of Internet tools has a restrictive influence on the execution of the usual strategy of solution.

P (Biotechnology application in Medicine)

Antimicrobial Compounds from plants -Biotechnology application in Medicine

Literature Review

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Background: Finding healing powers in plants is an ancient idea. It is known that Neanderthals used specific parts of plants to cure infectious conditions. Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found in vitro to have antimicrobial properties. Clinical microbiologists have many reasons to investigate those bioactive compounds (new antibiotics, numerous resistant to common antibiotics bacteria, less harmful chemical compounds etc).

Methods: We conducted a literature search using PubMed, MEDLINE, complemented by Google Scholar search using key words of plant bioactive compounds, essential oils, antimicrobial activity, oil extraction, assays and plants used for biotechnology purposes (for human health). We identified original research articles published during 2003-2013 in English language.

Results: There were 708 original research articles investigating the essential oils of plants, 168 investigating the bioactive compounds and 37 investigating methods of extractions. Bioactive compounds may be a result of constituent expressed genes or may be a result of stressful conditions. At least 12,000 secondary metabolites have been isolated, a number estimated to be less than 10% of the total.

Conclusions: The literature indicated that there are many researches about plants' bioactive compounds. Plant oils are well known for their antimicrobial activity. Modern biotechnology plays a crucial role in the development of new diagnostic methods and better targeted drugs. Substances such as essential oils should attract the interest of researchers and pharmaceutical companies for clinical studies and other applications in the therapy of diseases.

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1^η Προφορική ανακοίνωση

Επεξεργασία σκελετικού μυός

Σπύρος Καλαντζάκης

Τεχν. Ιατρ. Εργαστηρίων. Τμήμα Παθολ. Ανατομικής. Νοσοκομείο Κοργιαλένιο-Μπενάκειο

Εισαγωγή. Ο ανθρώπινος οργανισμός αποτελείται από: Τους λείους μύες, τον καρδιακό μύ, και τους σκελετικούς μύες (Αποτελούν το 40-45% της μάζας του σώματος)

Οι φυσιολογικοί μύες αποτελούνται από μυϊκές ίνες, ομαδοποιημένες σε δεσμίδες και περιβαλλόμενες από συνδετικό ιστό που ονομάζεται περιμύϊο.

Κάθε μυϊκή ίνα είναι ένα επιμηκυσμένο πολυπύρρνο κύτταρο προερχόμενο εμβρυολογικά από την σύντηξη εκατοντάδων μυοβλαστών. Επειδή το μήκος του μυοκυττάρου είναι μεγάλο, προτιμάται ο όρος μυϊκή ίνα.

Σκοπός. Η διάγνωση διαφόρων μυοπαθειών όπως: μυοσίτιδες, μυϊκές δυστροφίες, συγγενείς ή μιτοχονδριακές μυοπάθειες, κλπ.

Υλικά και μέθοδος. Συνήθως από τον δικέφαλο ή τον τετρακέφαλο μυ. Διαστάσεις μυϊκής βιοψίας: έως (0,5X0,5X1) cm. Τοποθέτηση σε πλαστικό δοχείο που περιέχει βρεγμένη γάζα με ελάχιστο φυσιολογικό ορό έτσι ώστε να διατηρείται ο μυς νωπός. Άμεση αποστολή στο παθολογοανατομικό εργαστήριο (εντός 1 ώρας). Για να διατηρηθεί η ενζυμική δραστηριότητα του μυός πρέπει να τον παγώσουμε με τέτοιο τρόπο ώστε να μονιμοποιηθούν και να ψυχθούν σε πολύ σύντομο χρονικό διάστημα οι μυϊκές ίνες.

Πρώτα βάζουμε υγρό άζωτο στο ειδικό δοχείο. (-170 βαθμοί Κελσίου).

Στο ειδικό πλαστικό φιαλίδιο βάζουμε ισοπεντάνιο τόσο ώστε να μπορέσει να καλύψει πλήρως την μυϊκή βιοψία που θα εμβαπτίσουμε μέσα. Το φιαλίδιο αυτό θα τοποθετηθεί μέσα στο ειδικό δοχείο που περιέχει το υγρό άζωτο έως ότου ασπρίσουν τα τοιχώματα. (Σημείο τήξης του ισοπεντανίου -160 βαθμοί Κελσίου). Εμβαπτίζουμε την μυϊκή βιοψία για 10 δευτερόλεπτα μέσα στο ισοπεντάνιο που έχει μόλις αρχίσει να τήκεται και ανακινούμε ελαφρά. Ο προσανατολισμός του δείγματος και η ομοιόμορφη φορά των μυϊκών ινών είναι απαραίτητη για την σωστή λήψη των τομών στον ψυκτικό μικροτόμο μετέπειτα. Τοποθετούμε γρήγορα τον κατεψυγμένο μυ σε φιαλίδιο corning και όλο μαζί στο υγρό άζωτο και κατόπιν στο ειδικό δοχείο φύλαξης κατεψυγμένων δειγμάτων.

Τομές στον ψυκτικό μικροτόμο:

Φορά κοπής: Κάθετα ως προς τον άξονα των μυϊκών ινών.

Θερμοκρασία κρουστάτη: -25 έως -30 βαθμούς Κελσίου.

Πάχος τομών: 10 -12 μm

Αποτελέσματα και συμπεράσματα. Με τις παρακάτω χρώσεις:

Ενζυμικές: COX, SDH, Phosphorylase, NADH, κλπ

Ιστοχημικές: Gomori, PAS κλπ

Ανοσοϊστοχημικές: Dystrofin 1,2,3, Merosin κλπ

Χρωματίζονται οι διαφορετικού τύπου μυϊκές ίνες (τύπου α και β) δίνοντας την δυνατότητα στον ιατρό Παθολογοανατόμο να κάνει την τελική του διάγνωση.

2^η Προφορική ανακοίνωση

Επιπλασμός της μετάλλαξης A1298C του γονιδίου της 5,10-μεθυλενο-τετραυδροφυλλικής αναγωγάσης (MTHFR) σε νεαρά άτομα με ιστορικό συγγενούς θρομβοφιλίας

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Εισαγωγή. Η 5,10-μεθυλενοτετραϋδροφυλλική αναγωγή (MTHFR) είναι ένα σημαντικό ένζυμο στο μεταβολισμό του φυλλικού οξέος. Μεταλλάξεις στη λειτουργική περιοχή του ενζύμου δύναται να επηρεάσουν τη δραστικότητα του και το μεταβολισμό του φυλλικού οξέος, με αποτέλεσμα την παθογένεια ορισμένων διαταραχών, μια εκ των οποίων είναι η εμφάνιση θρομβοεμβολικών επεισοδίων. Η σημειακή μετάλλαξη A1298C, είναι μία εκ των δύο πιο συχνών μεταλλάξεων του γονιδίου MTHFR και έχει συσχεισθεί με ήπια υπερομοκυστεϊναιμία. Οι ομοζυγώτες για την εν λόγω μετάλλαξη, εμφανίζουν μειωμένη ενζυμική δραστικότητα κατά 41% σε σχέση με το φυσιολογικό πληθυσμό.

Σκοπός. Η παρούσα μελέτη ως σκοπό είχε αφενός τη διερεύνηση της συχνότητας της μετάλλαξης A1298C του γονιδίου MTHFR και αφετέρου τη συσχέτιση του εν λόγω πολυμορφισμού με τα επίπεδα της ομοκυστεϊνης στο πλάσμα, σε νεαρά άτομα με ιστορικό συγγενούς θρομβοφιλίας.

Υλικό και Μέθοδοι. Υλικό της μελέτης αποτέλεσαν 67 δείγματα αίματος που ελήφθησαν από νεαρά ως επί το πλείστον άτομα (55 γυναίκες, 12 άνδρες), με μέσο όρο ηλικίας τα 26 έτη. Πληροφορίες που αφορούσαν σε δημογραφικά δεδομένα καθώς και στο ιστορικό συγγενούς θρομβοφιλίας σε βάθος τριών γενεών, συλλέχθηκαν μέσω ερωτηματολογίου που διανεμήθηκε. Τα DNA απομονώθηκαν από τα δείγματα του περιφερικού αίματος και για τον εντοπισμό του πολυμορφισμού A1298C εφαρμόστηκε η μέθοδος ανάλυσης PCR-RFLP. Τα επίπεδα ομοκυστεϊνης στο πλάσμα προσδιορίστηκαν στο αυτόματο μηχανήμα ηηκτικού μηχανισμού ACL Advance.

Αποτελέσματα. Τα αποτελέσματα της μελέτης κατέδειξαν ομοζυγωτία για το φυσιολογικό γονότυπο (AA) σε 34 δείγματα (50.7%) και ετεροζυγωτία (AC) σε 33 δείγματα (49.3%). Ο ομόζυγος γονότυπος CC, δεν ανιχνεύθηκε. Η συχνότητα των αλληλίων σύμφωνα με τα αποτελέσματά μας ήταν 0,753 για το αλληλόμορφο A και 0,247 για το αλληλόμορφο C. Η συχνότητες των γονοτύπων για τον A1298C πολυμορφισμό δε συνάδουν με το ισοζύγιο Hardy-Weinberg, καθώς δεν υπάρχει ο γονότυπος CC ($P < 0,05$).

Συμπεράσματα. Η απουσία ομοζυγώτη 1298CC στα αποτελέσματά μας, πιθανώς σχετίζεται με το στατιστικά σχετικά μικρό δείγμα μελέτης σε συνδυασμό με τη χαμηλή συχνότητα του CC γονοτύπου στον πληθυσμό. Συμπεριλαμβάνοντας τις τιμές της ομοκυστεϊνης πλάσματος στην αξιολόγησή μας, δεν προκύπτει συσχέτιση αυξημένων επιπέδων αυτής με την ύπαρξη του μεταλλαγμένου αλληλομόρφου, καθώς τα επίπεδα την ομοκυστεϊνης στα ομόζυγα (AA) άτομα κυμαινόταν μεταξύ 4,4-36,7 $\mu\text{mol/L}$ ενώ στους ετεροζυγώτες (AC) μεταξύ 5,8-21,9 $\mu\text{mol/L}$. Μελέτη μεγαλύτερου δείγματος θα οδηγήσει σε περισσότερο ασφαλή συμπεράσματα.

3^η Προφορική ανακοίνωση

Εθνικό πρόγραμμα ελέγχου νεογνών (Ε.Π.Π.Ε.Ν.): επιδημιολογική διερεύνηση ενζυμικών τύπων γαλακτοζαιμίας 2006-2012

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Ινστιτούτο Υγείας του Παιδιού - Δ/ση Ενδογενών Μεταβολικών Νοσημάτων.

Εισαγωγή. Η Γαλακτοζαιμία είναι ένα μεταβολικό νόσημα που κληρονομείται με τον υπολειπόμενο σωματικό γόνο και ανιχνεύεται στα πλαίσια του Εθνικού Προγράμματος Προληπτικού Ελέγχου Νεογνών (Ε.Π.Π.Ε.Ν.). Τα τρία ένζυμα που συμμετέχουν στο μεταβολισμό της Γαλακτόζης/Λακτόζης είναι η ουριδυλ-τρανσφεράση (GALT), η Γαλακτοκινάση (GALK), και η Ουριδυν-επιμεράση (UDP-GALE). Ανεπάρκεια ενός από τα προαναφερθέντα ένζυμα προκαλεί τη νόσο Γαλακτοζαιμία. Η κλασική μορφή της σχετίζεται με την έλλειψη ή ανεπάρκεια του ενζύμου ουριδυλ-τρανσφεράση (GALT) και προκαλεί θορυβώδη συμπτώματα, ενώ η ανεπάρκεια των άλλων δύο σχετίζεται με ηπιότερες μορφές της νόσου.

Σκοπός. Είναι ο εντοπισμός, ο ενζυμικός προσδιορισμός της έλλειψης ή ανεπάρκειας, η αντιμετώπιση και η παρακολούθηση των πασχόντων νεογνών από τη νόσο Γαλακτοζαιμία.

Υλικά και μέθοδοι. Ελέγχθηκαν 713.057 νεογνά ηλικίας 3-5 ημερών και μετρήθηκαν τα επίπεδα Ολικής Γαλακτόζης (ΟΓ) σε αποξηραμένες σταγόνες αίματος σε κάρτα Guthrie. Ο προσδιορισμός της Ο.Γ. έγινε με φωτομετρική μέθοδο. Σε κάθε νεογνό με τιμή Ο.Γ. από 7 έως 15 mg/dl ζητήθηκε επανάληψη με δεύτερη κάρτα Guthrie. Με τιμή Ο.Γ. μεγαλύτερη από 15 mg/dl ζητήθηκε δεύτερη κάρτα Guthrie, βιοχημικός ηπατικός έλεγχος, ποσοτικός προσδιορισμός αμινοξέων ορού για τον αποκλεισμό τυχούσης

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αμινοξεοπάθειας και οφθαλμολογικός έλεγχος. Ο θηλασμός ή η σίτιση του νεογνού με άλλο φυσικό γάλα αντικαταστάθηκε με γάλα ελεύθερο Λακτόζης/Γαλακτόζης μέχρι την εξαγωγή των τελικών αποτελεσμάτων μετά από 36-48 ώρες. Ο προσδιορισμός της δραστηριότητας της ουριδουλ-τρανσφεράσης έγινε στο εργαστήριο μας με φθοριομετρική μέθοδο, ενώ των ενζύμων επιμεράσης, γαλακτοκινάσης καθώς και οι προσδιορισμοί DUARTE 1 και DUARTE 2 έγιναν στο Πανεπιστήμιο του Μονάχου.

Αποτελέσματα. Στο συγκεκριμένο χρονικό διάστημα βρέθηκαν συνολικά σαράντα νεογνά με Γαλακτοζαιμία

Έτος	Αριθμός νεογνών	Ενζυμικός τύπος			
		GALT	GALK	GALE	Duarte
2006 β' εξάμηνο	50720	1	0	0	2
2007	108526	1	0	1	7
2008	117304	3	1	1	2
2009	116859	4	0	0	1
2010	113323	1	1	0	1
2011	105096	3	4	1	0
2012	101229	1	1	1	2
ΣΥΝΟΛΟ	713057	14	7	4	15
Συχνότητες		1:50933	1:101865	1:178264	1:47537

1:17826 νεογνά με διαταραχές στο μεταβολισμό της Γαλακτόζης.

Συμπεράσματα. Η συχνότητα εμφάνισης της Κλασικής Γαλακτοζαιμίας είναι ίδια με εκείνη που αναφέρεται σε άλλους Ευρωπαϊκούς Πληθυσμούς, όπως επίσης και εκείνη της ανεπάρκειας της Επιμεράσης. Όμως, η συχνότητα εντοπισμού ανεπάρκειας της Γαλακτοκινάσης είναι υψηλότερη συγκριτικά με εκείνες που αναφέρονται στη Διεθνή Βιβλιογραφία. Οι τύποι Duarte 1 και Duarte 2 είναι οι συχνότερες μορφές που εντοπίζονται με τον Ε.Π.Π.Ε.Ν. όπως συμβαίνει σε όλα τα προγράμματα διερεύνησης Γαλακτοζαιμίας.

Βιβλιογραφία. SchulpisKH, PapakonstantinouE, MichelakakisH, PodskarbiT, PatsouraA, Shin 4. Screening for galactosemia in Greece. *Pediatr Perinatal Epidemiol* 1997, 11:436-440, Diepenbrock Fr, Heckler H, Schicking J, Engelharod T, Bock D, Sande J, Cororimetric determintion of galactose and galactose-1-phosphate from dried blood. *Clinical Biochemistry* 1992: 25:37-39.

4^η Προφορική ανακοίνωση

Η επίδραση της χρήσης χλωρίου στην απολύμανση έτοιμων προς κατανάλωση τροφίμων

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Τα φρέσκα φρούτα και τα λαχανικά μπορεί να περιέχουν υψηλά επίπεδα παθογόνων μικροοργανισμών μετά την συγκομιδή τους, με αποτέλεσμα την πιθανότητα πρόκλησης τροφικών δηλητηριάσεων. Μερικοί μικροοργανισμοί που προκαλούν τροφιογενείς επιδημίες είναι *Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis* και *Listeria monocytogenes* (Ölmezetal., 2009). Επομένως, η απολύμανση κρίνεται αναγκαία για την αποφυγή τέτοιων επιδημιών. Το χλώριο αποτελεί το πιο κοινό και ευρέως χρησιμοποιούμενο απολυμαντικό από τις βιομηχανίες τροφίμων. Στη παρούσα μελέτη, τρία είδη τροφίμων: μαρούλια, ντομάτες τύπου cherry και φράουλες λόγω αυξημένης παραγωγής στην Ελλάδα, επιλέχθηκαν και εμβολιάσθηκαν με μίγμα τεσσάρων μικροοργανισμών (*Escherichia coli*, *Staphylococcus aureus*, *Salmonella enteritidis* και *Listeria monocytogenes*). Στη συνέχεια πραγματοποιήθηκε επεξεργασία των τροφίμων με υποχλωριώδες νάτριο διαφορετικών συγκεντρώσεων (50 και 200 ppm) και σε διαφορετικούς χρόνους επαφής (1,3,5 λεπτά). Όλοι οι μικροοργανισμοί και στα τρία τρόφιμα

παρουσίασαν σταθερή μείωση σε όλους τους χρόνους επεξεργασίας. Η λογαριθμική μείωση των μικροοργανισμών στο πρώτο λεπτό επεξεργασίας με NaOCl 50ppm και 200ppm κυμάνθηκε στο μαρούλι από 1,5-2 log CFU/g και 1,6-3 log CFU/g αντίστοιχα. Στα ντοματίνια 0,59-1,65 log CFU/g και 0,95-1,89 log CFU/g. Τέλος, στις φράουλες 0,20-1,4 log CFU/g και 0,22-1,52 log CFU/g αντίστοιχα. Παρατηρήθηκε ότι οι διαφορετικές συγκεντρώσεις χλωρίου δεν έχουν σημαντικά στατιστικό ρόλο στη μείωση του βακτηριακού πληθυσμού ενώ σημαντικό ρόλο έχει ο χρόνος επαφής. Επιπλέον βρέθηκε ότι σε τρόφιμα με πορώδη επιφάνεια (φράουλα) η αποτελεσματικότητα του χλωρίου δεν ήταν ικανοποιητική.

Συμπεράσματα: Συνεπώς, για ένα καλύτερο δυνατό αποτέλεσμα στην εξάλειψη των παθογόνων μικροοργανισμών θα ήταν εφικτό να δοκιμαστούν συνδυασμοί χλωρίου χαμηλής συγκέντρωσης με νέες μη θερμικές τεχνολογίες απολύμανσης (π.χ. υπεριώδης ακτινοβολία, υπέρηχοι, παλλόμενα ηλεκτρικά πεδία κ.α.) (Birmpra et al., 2013, Goodburn et al., 2013) με σκοπό την επιτυχή απολύμανση των τροφίμων σε σύντομο χρονικό διάστημα.

5^η Προφορική ανακοίνωση

Εργασιακά ατυχήματα που καταλήγουν σε λοίμωξη σε νοσοκομεία της Ελλάδας αστικού συγκροτήματος μεσαίας δυναμικότητας

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Εισαγωγή. Έκαστος νοσοκομειακός οφείλει να δίνει την πρέπουσα σημασία στην ατομική του καθαριότητα και υγιεινή, να τηρεί πιστά τους κανονισμούς ασφαλείας χώρων και μηχανημάτων, αλλά και να ειδοποιεί τους υπεύθυνους για τυχόν κινδύνους, που αντιλαμβάνεται και να απαιτεί στη συνέχεια την λήψη μέτρων ασφαλείας, όπου αυτά λείπουν ή είναι πλημμελή (Λινού Α., Ιατρική της Εργασίας, Εκδόσεις Βήτα, (c) 2005-Βελονάκης Μ., Τσαλίκογλου Φ., Συστήματα διαχείρισης υγείας και ασφάλειας κατά της εργασίας σε νοσοκομείο. Εκδόσεις Παρισιάνου, 2005-Παπαδόπουλος Γ., Καλοβούλου Λ., Σοφός Α., Νοσοκομειακές λοιμώξεις, Επιδημιολογία, Πρόληψη, Έλεγχος, Εκδόσεις Παρισιάνου, 1997).

Σκοπός. Να καταδειχθεί το ενδεχόμενο ενδονοσοκομειακής λοίμωξης στο Προσωπικό νοσηλευτικών ιδρυμάτων, κατόπιν εργασιακού ατυχήματος.

ΥΛΙΚΑ-ΜΕΘΟΔΟΣ: Χρησιμοποιήθηκε ελληνική ιατρική βιβλιογραφία και ερωτηματολόγιο 30 ερωτήσεων, που μοιράστηκε στο σύνολο των Εργαστηριακών (Μικροβιολογικά, Αιματολογικά, Βιοχημικά και Ανοσολογικά) τριών Νοσοκομείων Θεσ/νίκης (Θεαγένειο, Λοιμωδών και Κεντρικό). Διήρκεσε η διαδικασία 5 μήνες (1/10/2012-31/12/2012 & 1/3/2013-31/5/2013) και απαντήθηκαν 180 σε σύνολο 194 ερωτηματολογίων, που μοιράστηκαν στις ειδικότητες: Ιατροί, Βιολόγοι, Τεχνολόγοι Ιατρικών Εργαστηρίων, Παρασκευαστές, Ειδικευόμενοι και Ασκούμενοι.

Αποτελέσματα. Στο σύνολο των τριών νοσοκομείων καταγράφηκε 20,46% των ερωτηθέντων, να δηλώνουν πως ουδέποτε παρατηρήθηκε ενδονοσοκομειακή λοίμωξη ακολουθούμενη εργασιακού ατυχήματος. Σπάνιο τέτοιο περιστατικό σημειώνει το 56,82% του συνόλου και σε μέτριο βαθμό το 19,32%. Συχνή ενδεχόμενη λοίμωξη υπογραμμίζει το 2,28%. Προχωρώντας σε σύγκριση μεταξύ των νοσοκομείων, αξίζει να τονιστεί πως το μεγαλύτερο ποσοστό για συχνή ενδονοσοκομειακή λοίμωξη κατόπιν επαγγελματικού ατυχήματος εμφανίζεται στο «Κεντρικό» (5%), ποσοστό που μηδενίζεται κυριολεκτικά στο «Λοιμωδών». Οι περισσότερες ανύπαρκτες τέτοιες περιπτώσεις κατά δήλωση του Προσωπικού βρίσκονται στο «Κεντρικό» (30%), ενώ μειώνονται στο «Θεαγένειο» (15,09%). Στα σπάνια τέτοια ενδεχόμενα δεν καταγράφονται ουσιαστικές διαφοροποιήσεις μεταξύ των νοσοκομείων (55-58,49%). Τέλος περιστατικά σε μέτριο βαθμό συγκριτικά πλεονεκτούν στο «Θεαγένειο» (22,64%) και σαφώς λιγότερα σημειώνονται στο «Κεντρικό» (10%).

Συμπεράσματα. Δεδομένου των υποδομών που ισχύουν στα περισσότερα νοσοκομεία μας, κρίνεται ελπιδοφόρο το γεγονός, πως σε σημαντικό ποσοστό (78,4%) δεν ταλαιπωρούνται τα υγειονομικά στελέχη

και οι υπάλληλοι από ενδονοσοκομειακές λοιμώξεις ως αποτέλεσμα επαγγελματικού ατυχήματος. Σημαντικό ρόλο επίσης φαίνεται να παίζει και η φύση του νοσηλευτικού ιδρύματος, καθότι εξειδικευμένα νοσοκομεία σε λοιμώξεις μηδενίζουν τα συχνά τέτοια περιστατικά, που σημαίνει ότι το Προσωπικό τους συμπεριφέρεται με ιδιαίτερη προσοχή στην άσκηση των καθηκόντων του. Υπάρχει ωστόσο και μεγάλο περιθώριο βελτίωσης, προκειμένου να αγγίξουμε το άριστον, αφού δεν μπορεί να σημειωθεί ως ευκαταφρόνητο το 21,6%, που δηλώνει περιπτώσεις ενδονοσοκομειακής λοίμωξης σε μέτριο έως συχνό βαθμό κατόπιν εργασιακού ατυχήματος.

6^η Προφορική ανακοίνωση

Διαχείριση αποβλήτων σε τριτοβάθμια υγειονομική μονάδα.

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Εισαγωγή: Υπάρχει ένα γενικότερο παγκόσμιο πρόβλημα με τη διαχείριση των απορριμμάτων, πρόβλημα που γίνεται εντονότερο με τη διαχείριση των αποβλήτων υγειονομικών μονάδων τα οποία είναι και επικίνδυνα για την Δημόσια Υγεία

Σκοπός: Η δημοσιοποίηση του μεγέθους των παραγομένων αποβλήτων από τριτοβάθμιο νοσοκομείο στους συναδέλφους επαγγελματίες υγείας, η μέθοδος και το κόστος διαχείρισής τους ανά είδος, με στόχο την ευαισθητοποίηση των συναδέλφων για την σωστή και στον τόπο παραγωγής αυτών.

Υλικό και μέθοδος: Χρησιμοποιήθηκαν τα επίσημα στοιχεία από τριτοβάθμιο νοσοκομείο της Αθήνας που εστάλησαν στο Υπουργείο Περιβάλλοντος για το έτος 2012. Η επεξεργασία στοιχείων έγινε με Microsoft Excel.

Αποτελέσματα: Στο νοσοκομείο παρήχθησαν το έτος 2012, 1.092 τόνοι αστικών αποβλήτων τα οποία μετεφέρθησαν από τις υπηρεσίες του Δήμου στους χώρους υγειονομικής ταφής. Σε αυτά συμπεριλαμβάνονταν χαρτί, χαρτόνια, γυαλιά και αλουμίνιο. Ακόμη 177.000 kg επικινδύνων αποβλήτων αμιγώς μολυσματικών τα οποία διαχειρίστηκαν με την μέθοδο της αποστείρωσης με κόστος 1.23€/kg και 24.500kg επικινδύνων (μολυσματικών, τοξικών και άλλων) αποβλήτων τα οποία διαχειρίστηκαν με την μέθοδο της αποτέφρωσης με κόστος 2,00€/kg. Ανακυκλώθηκαν περίπου 400 kg αλκαλικών μπαταριών και μεγάλων συσσωρευτών, 1.000 Kg ηλεκτρικού και ηλεκτρονικού εξοπλισμού, 380 Kg λαμπτήρες πυρακτώσεως, 460kg από ηλεκτρονικό εξοπλισμό που περιείχε επικίνδυνα στοιχεία. Από την ανακύκλωση το νοσοκομείο είχε μηδενικά έσοδα.

Συμπεράσματα: Υπάρχει αυξημένο κόστος των αποβλήτων που απαιτούν ειδική διαχείριση (αποστείρωση, αποτέφρωση) συγκριτικά με τα αστικά απόβλητα. Επομένως όπου είναι εφικτό θα πρέπει να υπάρχει προσπάθεια σωστού διαχωρισμού στην πηγή παραγωγής. Έτσι θα μπορούσε να μειωθεί το κόστος διαχείρισης, ενώ η ανακύκλωση συσκευασιών (αλουμινίου, χαρτιού, υλικών συσκευασίας) θα μπορούσε να αποφέρει αφενός έσοδα στο νοσοκομείο (για χρήση πχ σε κοινωνικούς σκοπούς), ενώ θα δίνει και την ικανοποίηση της συμμετοχής- ανταμοιβής στην ομάδα της ανακύκλωσης από τους εργαζόμενους.

7^η Προφορική ανακοίνωση

Κρούσματα ηπατίτιδας C κατά τα έτη 1998 – 2011 στην Ελλάδα

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Εισαγωγή: Η Ηπατίτιδα C είναι ένα λοιμώδες νόσημα, το οποίο προκαλείται από τον ιό HCV (Hepatitis C Virus). Ο ιός προσβάλλει τα ηπατοκύτταρα και προκαλεί κίρρωση και ηπατοκυτταρικό καρκίνωμα. Το γενετικό υλικό του ιού είναι μονόκλωνο RNA. Ηπατίτιδα μπορούν να προκαλέσουν και άλλοι ιοί, οι οποίοι ονομάζονται ηπατοτρόποι. Αυτοί εκτός από το ήπαρ προσβάλλουν και άλλα όργανα.

Σκοπός: Σκοπός της συγκεκριμένης εργασίας, είναι να γίνει μια καταγραφή όλων των κρουσμάτων Ηπατίτιδας C στην Ελλάδα, όπως αυτά έχουν καταγραφεί στη βάση δεδομένων του Κέντρου Ελέγχου και

Πρόληψης Νοσημάτων (ΚΕ.ΕΛ.Π.ΝΟ.). Επιπρόσθετα, θα γίνει μια σύγκριση με τα κρούσματα, τόσο στην Αμερική, όσο και παγκοσμίως, όπως αυτά καταγράφηκαν στη βάση δεδομένων: Centers for Disease Control and Prevention (www.cdc.gov).

Υλικά και Μέθοδοι: Στη συγκεκριμένη μελέτη χρησιμοποιήθηκαν τα στοιχεία που είναι καταγεγραμμένα στο ενημερωτικό δελτίο του ΚΕ.ΕΛ.Π.ΝΟ (Μάρτιος 2012, αφιερωμένο στις Ηπατίτιδες) καθώς και στη βάση δεδομένων του Centers for Disease Control and Prevention, για τα κρούσματα των ΗΠΑ και διεθνώς. Τα στοιχεία που συγκεντρώσαμε αφορούν τον επιπολασμό ανά γεωγραφικό διαμέρισμα, τη μέση τιμή περιστατικών ανά έτος από το 2004 - 2011, την επίπτωση της Ηπατίτιδας C ανά 100.000 ανθρώπους για τα έτη 1998 – 2011.

Αποτελέσματα: Ο επιπολασμός της Ηπατίτιδας C είναι αρκετά υψηλός στην Αττική. Αυτό συμβαίνει, γιατί είναι άρρηκτα δεμένη με τη μετανάστευση καθώς και για το γεγονός ότι υπάρχουν περισσότερα νοσοκομεία στο γεωγραφικό αυτό διαμέρισμα. Όσον αφορά τις ηλικίες, έξαρση τα τελευταία χρόνια παρουσιάζει η ηλικιακή ομάδα από 40 - 59 ετών. Υψηλό ποσοστό κρουσμάτων, εμφανίζουν τα άτομα με αλλοδαπή υπηκοότητα καθώς και οι ασθενείς που έχουν κάνει πρόσφατα χειρουργική επέμβαση. Στη δεύτερη θέση βρίσκονται άτομα που κάνουν αιμοκάθαρση ή μετάγγιση. Τα δεδομένα είναι ίδια και για την ασυμπτωματική Ηπατίτιδα C, καθώς τα άτομα με αλλοδαπή υπηκοότητα και οι πρόσφατα χειρουργημένοι ασθενείς συγκεντρώνουν μεγάλα ποσοστά.

Συμπεράσματα: Τα τελευταία χρόνια, παρατηρείται μια έξαρση της Ηπατίτιδας C, τόσο στην Ελλάδα, όσο και διεθνώς. Αυτό οφείλεται πρώτον στην απουσία εμβολίου (είναι ακόμα σε πειραματικό επίπεδο) καθώς και στην αμέλεια των ασθενών να πάρουν φάρμακα. Αν λάβουμε υπόψη και τη συλλοίμωξη με τον ιό HIV, τότε τα πράγματα γίνονται ακόμη πιο δύσκολα για τη θεραπεία, επειδή η μία λοίμωξη επηρεάζει την πορεία της άλλης. Η Ηπατίτιδα C δεν θεραπεύεται και μεταπίπτει σε χρόνια μορφή, οδηγώντας τους ασθενείς σε θάνατο, είτε από καρκίνο, είτε από κάποια άλλη συλλοίμωξη.

8^η Προφορική ανακοίνωση

Κρούσματα ηπατίτιδας B κατά τα έτη 1998 – 2011 στην Ελλάδα

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Εισαγωγή: Η Ηπατίτιδα είναι ένα λοιμώδες νόσημα που προκαλείται από τον ιό HBV (Hepatitis B Virus). Το γενετικό υλικό του ιού αυτού είναι δίκλωνο DNA. Προσβάλλει τα ηπατικά κύτταρα, δημιουργώντας φλεγμονή. Αν μεταπέσει σε χρονιότητα οδηγεί σε κίρρωση ήπατος και ηπατοκυτταρικό καρκίνωμα. Επίσης υπάρχουν και άλλοι ιοί που δύνανται να προκαλέσουν ηπατίτιδα. Τους ιούς αυτούς τους ονομάζουμε ηπατοτρόπους και προσβάλλουν και άλλα όργανα εκτός του ήπατος.

Σκοπός: Σκοπός της συγκεκριμένης εργασίας είναι να γίνει μια καταγραφή όλων των κρουσμάτων Ηπατίτιδας B στην Ελλάδα, όπως αυτά έχουν καταγραφεί στην βάση δεδομένων του Κέντρου Ελέγχου και Πρόληψης Νοσημάτων (ΚΕ.ΕΛ.Π.ΝΟ.). Επιπρόσθετα θα γίνει μια σύγκριση με τα κρούσματα, τόσο στην Αμερική, όσο και παγκοσμίως, όπως αυτά καταγράφηκαν στη βάση δεδομένων: Centers for Disease Control and Prevention (www.cdc.gov).

Υλικά και Μέθοδοι: Στη συγκεκριμένη μελέτη χρησιμοποιήθηκαν τα στοιχεία που είναι καταγεγραμμένα στο ενημερωτικό δελτίο του ΚΕ.ΕΛ.Π.ΝΟ (Μάρτιος 2012, αφιερωμένο στις Ηπατίτιδες) καθώς και στη βάση δεδομένων του Centers for Disease Control and Prevention για τα κρούσματα των ΗΠΑ και διεθνώς. Τα στοιχεία που συγκεντρώσαμε αφορούν τον επιπολασμό ανά γεωγραφικό διαμέρισμα, τη μέση τιμή περιστατικών ανά έτος από το 2004 - 2011, την επίπτωση της Ηπατίτιδας B ανά 100.000 ανθρώπους για τα έτη 1998 – 2011 καθώς και την επίπτωση ανά ηλικιακή ομάδα της οξείας Ηπατίτιδας B.

Αποτελέσματα: Ο επιπολασμός της Ηπατίτιδας B είναι αρκετά υψηλός στη Θράκη και τη Μακεδονία και αυτό συμβαίνει, γιατί είναι άρρηκτα δεμένη με τη μετανάστευση. Όσον αφορά στην ηλικιακή κατανομή, έξαρση Ηπατίτιδας B τα τελευταία χρόνια παρουσιάζει η ηλικιακή ομάδα από 25 – 49 ετών, ενώ έχει σχεδόν εκλείψει στις ηλικίες από 0 – 24 ετών. Υψηλό ποσοστό κρουσμάτων εμφανίζουν τα άτομα με αλλοδαπή υπηκοότητα καθώς και οι ασθενείς που έχουν κάνει οδοντοθεραπείες, να βρίσκονται στη δεύτερη θέση μαζί με τα άτομα που ανήκουν σε ομάδες υψηλού κινδύνου.

Συμπεράσματα: Η πρόληψη της Ηπατίτιδας Β έχει τεθεί ως προτεραιότητα από την παγκόσμια κοινότητα. Το εμβόλιο (χορηγείται σε τρεις δόσεις) δημιουργεί πολύ καλή ανοσοποίηση, γι' αυτό και έχουμε μείωση κρουσμάτων από τις ηλικίες 0 – 24 έτη τελευταία. Επίσης, τα μεγάλης ευαισθησίας τεστ που γίνονται στην αιμοδοσία έχουν μηδενίσει τις πιθανότητες να λάβει κάποιος μολυσμένο αίμα κατά τη μετάγγιση αίματος. Τα φάρμακα, σε περίπτωση που κάποιος νοσήσει, είναι πλέον εξειδικευμένα και μπορούν σε ορισμένες περιπτώσεις να εκριζώσουν τον ιό από τον οργανισμό.

9^η Προφορική ανακοίνωση

Βιολογικοί κίνδυνοι και επίπεδο ανοσοπροφύλαξης των εργαζομένων σε οδοντοτεχνικά εργαστήρια.

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Εισαγωγή. Στα οδοντοτεχνικά εργαστήρια, οι βιολογικοί παράγοντες κινδύνου είναι μια υποεκτιμημένη απειλή. Η αποφυγή των μολύνσεων είναι *ουσιαστική ευθύνη* της οδοντιατρικής, αλλά και της οδοντοπροσθετικής τεχνολογίας. Η δυνατότητα για μετάδοση ασθενειών στο οδοντοπροσθετικό εργαστήριο είναι επαρκώς τεκμηριωμένη. Οι παθογόνοι μικροοργανισμοί μπορούν να εισέλθουν στο εργαστήριο μέσω των αποτυπωμάτων, των οδοντικών προσθέσεων (δοκιμές - επιδιορθώσεις) και των συσκευών-εργαλείων ή και των εντύπων και γραπτών οδηγιών.

Στόχος: Στόχος της παρούσας εργασίας, είναι η ενημέρωση για τους κινδύνους μόλυνσης των ασθενών, αλλά και του προσωπικού των οδοντιατρείων και των οδοντοπροσθετικών εργαστηρίων κατά τη συνεργασία τους και της ανάγκης για ορθή εμβολιαστική κάλυψη του προσωπικού, ώστε να καταστεί το οδοντοπροσθετικό εργαστήριο όσο το δυνατόν ασφαλέστερο.

Σκοπός: Σκοπός αυτής της εργασίας, είναι η μελέτη της υποκειμενικής εκτίμησης των οδοντοτεχνιτών, ειδικότερα όσον αφορά τους βιολογικούς κινδύνους, την ανοσοπροφύλαξη των εργαζομένων και η εξαγωγή συμπερασμάτων καθώς και η διατύπωση προτάσεων για τη βελτίωση της επαγγελματικής τους υγείας.

Υλικά-Μέθοδοι: Για τη διερεύνηση αυτού του θέματος, διαμορφώθηκε πρωτότυπο ερωτηματολόγιο υποκειμενικής εκτίμησης, σύμφωνα με τα διεθνή πρότυπα, το οποίο διανεμήθηκε σε 230 οδοντοτεχνίτες της πόλης των Αθηνών, (ΜΠΣ «Υγιεινή και ασφάλεια στην εργασία», Τμήμα Ιατρικής ΔΠΘ, ΕΛΙΝΥΑΕ).

Αποτελέσματα: Οι 169 οδοντοτεχνίτες που απάντησαν είχαν μια μέση ηλικία τα 37,4 έτη(±11 έτη). Το 61,5% των συμμετεχόντων ήταν άντρες και το 38,5% γυναίκες, ενώ στη μελέτη του Γ. Δρακωνάκη ήταν 88,2% και 11,8% αντίστοιχα. Επίσης, σχετικά ίδια ποσοστά υπάρχουν και στη μελέτη των Rom και συν.1984 αλλά είναι σε αντίθεση με τη μελέτη των Sherson και συν. 1988 [18]. Το 51,2% των συμμετεχόντων ήταν καπνιστές και το ποσοστό αυτό είναι χαμηλότερο σε σχέση με τους ερωτηθέντες οδοντοτεχνίτες της Κρήτης, το οποίο ήταν 62,7%. Τα έτη προϋπηρεσίας στο συγκεκριμένο εργαστήριο ήταν 10,3 (±9,3), σε άλλα εργαστήρια ήταν 6,9 έτη (±6,4) και σε άλλη δουλειά ήταν 4,2 έτη (±5,2), ενώ κατά μέσο όρο το εβδομαδιαίο ωράριο εργασίας είναι: 45,0±10,4 ώρες. Σημαντικό είναι το εύρημα ότι, υπάρχουν εργαζόμενοι, οι οποίοι έχουν εργαστεί και σε καταστήματα υγειονομικού ενδιαφέροντος, όπως εστιατόρια και κρεοπωλεία. Το ποσοστό των υπαλλήλων οδοντοτεχνιτών είναι 79,8% με μέση τιμή±SD υπαλλήλων 6,9±6,3, το οποίο φαίνεται να συμφωνεί την έρευνα των Choudat D. et al. 1994 που αναφέρει μέσο όρο 2 και εύρος 1-12 υπαλλήλους. Το 75,1% των συμμετεχόντων, εργάζονται σε εργαστήρια γενικής προσθετικής κατά μέσο όρο 8,9 ώρες (±2) την ημέρα. Είναι αξιόλογο το εύρημα ότι, ενώ το 58,6% αναφέρει ότι το εργαστήριο, όπου εργάζεται απασχολεί τεχνικό ασφαλείας μόνο το 35,4% γνωρίζει τα MSDS των υλικών που χρησιμοποιεί, καθώς και ότι μόνο το 42,5% έχει εκπαιδευτεί σε θέματα υγιεινής και ασφαλείας της εργασίας. Ένα σημαντικό ποσοστό (74,7%), απάντησε ότι έχει συχνά/μερικές φορές πρόβλημα με το θόρυβο. Κατά την κατεργασία των μεταλλικών σκελετών

χρησιμοποιούν σπάνια/σχεδόν ποτέ ωτοασπίδες, το 95,2% των συμμετεχόντων. Το ποσοστό είναι σημαντικά υψηλότερο σε σχέση με το ποσοστό (15%) που αναφέρεται στη μελέτη των Jacobsen N. et al, 1996 που διενεργήθηκε σε Σουηδούς οδοντοτεχνίτες και είναι η μόνη μελέτη που περιλαμβάνει αρκετά θέματα υγιεινής των οδοντοτεχνιτών. Το γεγονός ότι το 69,6% απάντησε ότι έχει συχνά/μερικές φορές τρυπήματα και το 69,7% κοψίματα, είναι ιδιαίτερα σημαντικό καθώς τα τραύματα αποτελούν πύλη εισόδου παθογόνων μικροοργανισμών καθώς και τοξικών χημικών ουσιών. Το 97,6% αναφέρει ότι ο ρυθμός εργασίας είναι έντονος, γεγονός που συνοδεύεται και από υψηλό (88,6%) ποσοστό του υψηλού βαθμού ευθύνης. Είναι ενδεχόμενο να επηρεάζεται και η πνευματική κατάσταση (πνευματική κόπωση 64,8%), αλλά και να μειώνεται η προσοχή κατά την εργασία με συνέπεια τα αυξημένα ποσοστά τραυματισμών που παρατηρήθηκαν. Ένα σημαντικό ποσοστό 77,7% των οδοντοτεχνιτών, αναφέρει άγχος κατά την εργασία, αλλά και χαμηλή (58,2%) αναγνώριση της προσφοράς. Επιπλέον, το 57,4% των συμμετεχόντων, αναφέρει ότι δέχονται συχνά/ μερικές φορές πίεση από οδοντιατρεία, όταν δέχονται παραγγελίες. Διαπιστώνεται ότι η οδοντοτεχνική εργασία είναι μια στρεσογόνος εργασία. Τα ευρήματα αυτά, συμφωνούν με τη μελέτη των Park NG. et al.2003, η οποία διενεργήθηκε σε οδοντοτεχνίτες στην Κορέα. Στα συμπεράσματά τους αναφέρουν ότι, το καλύτερο επίπεδο οργάνωσης, συνεργασίας με το οδοντιατρείο, αλλά και διοίκησης της επιχείρησης, μπορεί να επιφέρει μείωση του επαγγελματικού στρες. Οι οδοντοτεχνίτες σε ποσοστό 12,1%, αναφέρουν ότι έχουν αλλεργία στο μεθακρυλικό μεθυλένιο, το οποίο ποσοστό είναι μικρότερο σε σχέση με το ποσοστό (21,3%) που αναφέρεται στην κλινική μελέτη της Ingrid E. et al. 2009. Είναι όμως σημαντικό το εύρημα ότι, το 17,8% των οδοντοτεχνιτών αναφέρει συμπτώματα δερματίτιδας-εκζέματος. Κατά την κατασκευή των ορθοδοντικών ακρυλικών το 91,7% των συμμετεχόντων, χρησιμοποιούν σπάνια/ σχεδόν ποτέ γάντια βινυλίου (προτείνονται για εργασία με ακρυλική ρητίνη). Κατά την κατασκευή των εργασιών με ακρυλική ρητίνη γενικά χρησιμοποιούν σπάνια/ σχεδόν ποτέ γάντια βινυλίου, το 94,8% των συμμετεχόντων. Σχετικά με την επίδραση του ακρυλικού στο νευρικό σύστημα, αναφέρονται ζαλάδες (33,3%) και η μελέτη των Fabrizio E. et al. 2007 [27], με τη διαπίστωση ότι επιβαρύνεται αρκετά το νευρικό σύστημα των οδοντοτεχνικών από την ανεξέλικτη έκθεση σε ατμούς και σκόνες μεθακρυλικού μεθυλενίου. Το 29,4% των συμμετεχόντων, εργάζονται σε εργαστήρια που υπάρχει ειδικός χώρος για την υποδοχή των αποτυπωμάτων και των εργασιών στα εργαστήρια. Το 43,9% των οδοντοτεχνιτών, απάντησε ότι μόνο το 25% των αποτυπωμάτων απολυμαίνονται στο εργαστήριο, ενώ μόνο το 20,6% αναφέρει ότι ο αριθμός των αποτυπωμάτων που παραλαμβάνονται από το οδοντιατρείο απολυμαίνονται στο εργαστήριο πάνω από 75%. Το 69,8% των συμμετεχόντων, απολυμαίνουν σπάνια/ σχεδόν ποτέ τους αρθρωτήρες και σε ποσοστό 65,5% σπάνια/ σχεδόν ποτέ απολυμαίνουν τα εργαλεία και τα μηχανήματα. Το 81,1% των συμμετεχόντων, πίστευαν ότι υπάρχει κίνδυνος λοίμωξης. Όμως, μόνο το 17,8% των συμμετεχόντων, έχουν κάνει την 1^η δόση του εμβολίου για τέτανο, το 11,2% τη 2^η και το 17,2%, την 3^η δόση. Επίσης, όσον αφορά στο εμβόλιο για την ηπατίτιδα Β, το 20,1% των συμμετεχόντων έχουν κάνει την 1^η και την 3^η δόση και το 17,2% τη 2^η δόση. **Συμπεράσματα:** Συνοψίζοντας, το 7,5% των συμμετεχόντων, έχουν υποβληθεί σε εργαστηριακό ή άλλο ιατρικό έλεγχο, ανάλογα με την επαγγελματική τους έκθεση. Τα ευρήματα αυτά είναι σημαντικά καθώς σε πολλές μελέτες, όπως των Vojdani M. et al. 2006 και των Verran J et al. 2004, τεκμηριώνεται ο κίνδυνος της μεταφοράς λοιμογόνων παραγόντων στο οδοντοτεχνικό εργαστήριο και ο κίνδυνος λοίμωξης των εργαζομένων.

Posters/Αναρτημένες Ελληνικές ανακοινώσεις

A (Μικροβιολογία) – 1

Επιδημιολογική μελέτη κρουσμάτων σαλμονέλας στην Ελλάδα κατά τα έτη 2004-2011.

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Εισαγωγή: Κυριότερη αιτία των τροφιμογενών νοσημάτων στην χώρα μας είναι η Σαλμονέλα του παράτυφου. Η μεγάλη ομάδα αυτή των gram (-) αρνητικών βακτηριδίων προσβάλλει τον άνθρωπο και μεταδίδεται μέσω πολλών εστιών αλλά οι σημαντικότερες είναι τα τρόφιμα και το νερό. Ειδικότερα τα τρόφιμα μολύνονται είτε από λάθος συντήρηση ή χειρισμούς είτε από πλύσιμο με μολυσμένο νερό.

Σκοπός: Σκοπός της συγκεκριμένης επιδημιολογικής μελέτης είναι να καταγραφούν όλα τα κρούσματα λοίμωξης από Σαλμονέλα κατά την επταετία 2004 - 2011 στην χώρα μας από μολυσμένα τρόφιμα ή νερό όπως αυτά έχουν καταχωρηθεί στην βάση δεδομένων του Κέντρου Ελέγχου και Πρόληψης Νοσημάτων (ΚΕ.ΕΛ.Π.ΝΟ).

Υλικά και Μέθοδοι: Υλικά για την συγκεκριμένη μελέτη αποτέλεσαν στοιχεία από την βάση δεδομένων του ΚΕ.ΕΛ.Π.ΝΟ. καθώς και από μερικές δημοσιευμένες εργασίες στην Ιατρική βάση άρθρων (pubmed.org). Βάση όλων αυτών έγινε μια στατιστική μελέτη τόσο για το πόσα άτομα νόσησαν από τα διάφορα στελέχη της Σαλμονέλας κατά την επταετία 2004-2011 όσο και για τον επιπολασμό ανά γεωγραφικό διαμέρισμα της χώρας μας. Δυστυχώς παρότι υπάρχουν οδηγίες από τον Ε.Φ.Ε.Τ. (Ενιαίος Φορέας Ελέγχου Τροφίμων) για τον σωστό χειρισμό και συντήρηση των τροφίμων, τα κρούσματα από Σαλμονέλα που αφορούν στα τροφιμογενή νοσήματα συνεχίζουν να είναι υψηλά.

Αποτελέσματα: Όσον αφορά στις συρροές η μέση ετήσια δηλούμενη επίπτωση στην Ελλάδα για την επταετία 2004 - 2011 ήταν: η μεγαλύτερη στα νησιά του Ιονίου με περίπου 9 συρροές ανά 1.000.000 κατοίκους ενώ η μικρότερη στην περιφέρεια Δυτικής Ελλάδας με περίπου 3 συρροές ανά 1.000.000 κατοίκους. Σε 326 περιπτώσεις, δηλαδή στο 75% περίπου των περιπτώσεων, ανευρέθηκε ο αιτιολογικός παράγοντας που προκάλεσε την λοίμωξη. Συνολικά κατά τα έτη αυτά δηλώθηκαν 268 συρροές κρουσμάτων σαλμονέλωσης.

Συμπεράσματα: Κατά την επιδημιολογική αυτή μελέτη διάφορα συμπεράσματα εξήχθησαν τα οποία μπορούν να συνοψιστούν στα εξής τρία:

1. Σωστή συντήρηση καθώς και πλύσιμο με τρεχούμενο νερό που προέρχεται από την εταιρία ύδρευσης και όχι από κάποιο πηγάδι.
2. Προσοχή ώστε να μην υπάρχει σύνδεση του αποχετευτικού δικτύου και του δικτύου ύδρευσης ώστε το νερό να είναι καθαρό και χωρίς βλαβερούς μικροβιακούς παράγοντες
3. Οποιαδήποτε λοίμωξη θα πρέπει να αναφέρεται, τόσο στον γιατρό όσο και στο Κέντρο Ελέγχου. Όταν πρόκειται για επιδημία θα πρέπει να αναφερθεί το περιστατικό και στον Ε.Φ.Ε.Τ.

Η μείωση των κρουσμάτων από τη σαλμονέλα είναι αποκλειστικά δική μας ευθύνη και πρέπει να εργαστούμε όλοι ώστε να εκλείψουν τα περιστατικά σαλμονέλωσης.

A (Μικροβιολογία) – 2

Ο ρόλος της δερμοαντίδρασης ΜΑΝΤΟΥΧ στη διάγνωση δευτερογενούς φυματίωσης

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Εισαγωγή: Η φυματίωση διακρίνεται στην πρωτογενή και δευτερογενή. Στην πρωτογενή ο ασθενής λαμβάνει ένα συνδυασμό αντιφυματικών φαρμάκων προκειμένου να αντιμετωπιστεί η νόσος. Στη δευτερογενή όμως, αμφισβητείται η χορήγηση αντιβιοτικών. Η δευτερογενής φυματίωση προβληματίζει ιδιαίτερα τους εργαζόμενους στο χώρο της υγείας. Η παρούσα βιβλιογραφική ανασκόπηση διερευνά αν η εξέταση mantoux βοηθά στη διάγνωση δευτερογενούς φυματίωσης.

Σκοπός: Η δερμοαντίδραση μαντού ή Tuberculin Skin Test (TST) είναι ένα δερματικό τεστ που βασίζεται στην ενδοδερμική ένεση μικρής ποσότητας φυματινικής πρωτεΐνης. Υπάρχουν διάφορα είδη φυματίνης. Στην Ελλάδα κυκλοφορεί η κεκαθαυμένη φυματίνη IP-48 (Institute Pasteur-48) που περιέχει 10 μονάδες (=5 TU PPD-S) ανά 0,1 ml. Η αντίδραση στο τεστ διαβάζεται μετά από 2-3 ημέρες. Στο σημείο έγχυσης της φυματίνης, παρατηρείται ερύθημα και σκληρία. Το αποτέλεσμα μετριέται σε χιλιοστά και αξιολογείται η μέγιστη εγκάρσια προς τον επιμήκη άξονα του αντιβραχίου διάμετρος της διήθησης (σκληρίας) και όχι της ερυθρότητας (κοκκινίλα). Είναι μια αντίδραση επιβραδυνομένου τύπου και για την αξιολόγηση λαμβάνονται υπόψη ποικίλοι παράγοντες οι οποίοι αναλύονται στην παρούσα εργασία.

Αποτελέσματα-συμπεράσματα: Μια θετική μαντού υποδηλώνει παρουσία μυκοβακτηρίου, μια αρνητική όμως δεν αποκλείει την παρουσία του, αφού μπορεί να παρατηρηθεί σε ποσοστό 20% των ατόμων που νοσούν. Η εξέταση περιπλέκεται καθώς μπορεί να δώσει ψευδώς θετικό ή αρνητικό αποτέλεσμα. Σύγχρονες μέθοδοι (πχ μέτρηση της ιντερφερόνης γ) πλεονεκτούν της δερμοαντίδρασης χωρίς όμως να είναι σε θέση να την αντικαταστήσουν.

A (Μικροβιολογία) – 3

Αίτια γαστρεντερίτιδας στα παιδιά το έτος 2012 στο Γ.Ν. Χανίων “Αγ. Γεώργιος”

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Εισαγωγή: Οι γαστρεντερίτιδες ιογενούς αιτιολογίας αποτελούν τη δεύτερη σε συχνότητα κλινική οντότητα μετά τις ιογενείς αναπνευστικές λοιμώξεις και το συχνότερο αίτιο διάρροιας σε παιδιά.

Σκοπός: Η επιδημιολογική διερεύνηση των γαστρεντερίτιδων στον παιδικό πληθυσμό που προσήλθε στο Γ. Ν. Χανίων κατά το έτος 2012.

Υλικά και μέθοδοι: Το υλικό μας αποτέλεσαν 707 δείγματα κοπράνων παιδιών με γαστρεντερίτιδα ηλικίας 30 ημερών μέχρι 14 ετών. Η καλλιέργεια για την αναζήτηση των εντεροπαθογόνων έγινε σε Mac Conkey, SS και Campylosel Agar και σε SS Agar κατόπιν ανακαλλιέργειας από ζωμό Selenite. Η ταυτοποίηση έγινε με τη χρήση των συμβατικών μεθόδων του εργαστηρίου. Η ανίχνευση των Rota και Adeno ιών επετεύχθη με τεστ ανοσοχρωματογραφίας ταχείας διάγνωσης.

ΑΠΟΤΕΛΕΣΜΑΤΑ:

Τρίμηνα έτους 2012	Σύνολο θετικών δειγμάτων	Rota virus	Adeno virus	Campylobacter spp	Salmonella spp
A	95	72	2	20	1
B	37	15	6	12	4
Γ	41	17	6	16	2
Δ	19	1	12	2	4
ΣΥΝΟΛΟ	192	105	26	50	11

Από τα συνολικά 707 δείγματα, θετικά για εντεροπαθογόνα βρέθηκαν τα 192 (27,2%). Από αυτά, 131 ήταν θετικά για Rota και Adeno ιούς (18,5%), ενώ οι βακτηριακές γαστρεντερίτιδες κατείχαν το 8,6 %.

Συμπέρασμα: Οι ιογενείς γαστρεντερίτιδες αποτελούν την πρώτη αιτία οξείας διάρροιας σε παιδιά, με κύριο αίτιο τους Rota ιούς (54,7%), με έξαρση κυρίως κατά το πρώτο τρίμηνο του έτους (χειμερινοί μήνες). Ακολουθούν οι βακτηριακές γαστρεντερίτιδες με κυριότερο αίτιο το Campylobacter spp σε ποσοστό 26% με την ίδια εποχιακή κατανομή.

A (Μικροβιολογία) – 4

Αναδρομική μελέτη μοριακής και φαινοτυπικής εργαστηριακής διάγνωσης μυκοβακτηριδιώσεων

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Εισαγωγή: Η φυματίωση προκαλείται από το *Mycobacterium tuberculosis complex* και αποτελεί σοβαρότατη, μεταδοτική νόσο που επιβάλλεται να διαγνωσθεί ταχέως. Άλλα είδη μυκοβακτηριδίων (άτυπα) ενδέχεται επίσης να προκαλέσουν λοίμωξη σε ανοσοκατασταλμένους ή ασθενείς με χρόνια πνευμονοπάθεια.

Σκοπός: Η αναδρομική μελέτη αποτελεσμάτων των μοριακών και φαινοτυπικών εξετάσεων ανίχνευσης μυκοβακτηριδίων στο Εργαστήριο Κλινικής Μικροβιολογίας του Π.Ν. «Αττικών» κατά το έτος 2012.

Υλικό-Μέθοδοι: Εξετάστηκαν 1.689 διαδοχικά δείγματα ασθενών. Μετά από κατεργασία με ακετυλοκουστεΐνη, εφαρμόστηκε η οξεάντοχη χρώση Ziehl-Neelsen και τα δείγματα καλλιεργήθηκαν σε σωληνάρια Loewenstein-Jensen και στο αυτοματοποιημένο σύστημα MGIT 960 (Becton Dickinson). Σε δείγματα με θετική οξεάντοχη χρώση καθώς και σε θετικά καλλιέργηματα εφαρμόστηκε η ταχεία μοριακή δοκιμασία MTBDRplus (PCR και υβριδισμός, Hain-Lifesciences) για την ταυτόχρονη ανίχνευση του *M. tuberculosis complex* και των μεταλλάξεων αντοχής σε ισονιαζίδη (INH) και ριφαμπικίνη (RIF). Στη συνέχεια πραγματοποιήθηκε ο φαινοτυπικός έλεγχος αντοχής (INH, RIF, στρεπτομυκίνη, εθαμβουτόλη και πυραζιναμίδη [MGIT 960]). Σε θετικό καλλιέργημα με αρνητική εξέταση MTBDRplus, εφαρμόστηκαν διαδοχικά οι μοριακές δοκιμασίες Genotype *Mycobacterium Common Mycobacteria* (CM) και *Additional Species* (AS) (Hain-Lifesciences) για ταυτοποίηση άτυπου μυκοβακτηριδίου (ταυτοποίηση 37 ειδών).

Αποτελέσματα: Βρέθηκε ότι 1.196 δείγματα προέρχονταν από το κατώτερο αναπνευστικό (70,8%), 158 αποτελούσαν πλευριτική συλλογή (9,3%) και 145 ήταν αιμοκαλλιέργειες (8,6%). Απομονώθηκαν μυκοβακτηρίδια από 50 ασθενείς. Σε 33 ασθενείς απομονώθηκε *M. tuberculosis complex* (συνολικά 80 δείγματα, εκ των οποίων 80% προέλευσης κατώτερου αναπνευστικού). Στους 25/33 ασθενείς (76%) το αποτέλεσμα της οξεάντοχης χρώσης ήταν θετικό και πραγματοποιήθηκε άμεση μοριακή διάγνωση της φυματίωσης και της αντοχής στην INH και RIF εφαρμόζοντας την δοκιμασία MTBDRplus (χρόνος λήψης αποτελέσματος 24 ώρες). Στους υπόλοιπους 8 ασθενείς, η οξεάντοχη χρώση ήταν αρνητική και η μοριακή δοκιμασία εφαρμόστηκε στο αντίστοιχο καλλιέργημα (μέσος χρόνος θετικοποίησης 17 μέρες, εύρος 6-33). Ο φαινοτυπικός έλεγχος αντοχής επιβεβαίωσε τα αποτελέσματα του μοριακού (1 ασθενής με στέλεχος INH-ανθεκτικό, 1 ασθενής με στέλεχος ταυτόχρονα INH/RIF-ανθεκτικό). Τέλος, από 17 ασθενείς απομονώθηκαν 8 είδη άτυπων μυκοβακτηριδίων με συχνότερα τα *Mycobacterium avium* και *Mycobacterium intracellulare*. Μόνο 3/17 ασθενείς (18%) παρουσίασαν θετικά αποτελέσματα οξεάντοχης χρώσης.

Συμπεράσματα: Οι μοριακές τεχνικές κάνουν δυνατή την ταχύτερη διάγνωση της φυματίωσης στο 76% των ασθενών, ανάμεσα στους οποίους περιλαμβάνονται όλοι αυτοί με τη μεταδοτικότερη μορφή της νόσου. Επιπλέον εξασφαλίζεται η έγκαιρη ανίχνευση της αντοχής και της πολυαντοχής. Τα άτυπα μυκοβακτηρίδια αποτελούσαν ικανό ποσοστό των απομονώσεων στον πληθυσμό που εξετάστηκε (34%), εν μέρει ως αποτέλεσμα της άρτιας μεθοδολογίας που εφαρμόστηκε.

A (Μικροβιολογία) – 5

Προσδιορισμός Θετικής Προγνωστικής Αξίας Εξετάσεων για τη Διάγνωση Οξείας Λοίμωξης από CMV και Τοξόπλασμα

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Εισαγωγή: Πρακτικοί λόγοι υπαγορεύουν την εφαρμογή διαδοχής εξετάσεων διαλογής και επιβεβαίωσης. Οι μέθοδοι διαλογής χαρακτηρίζονται από υψηλή ευαισθησία και ειδικότητα, αλλά η θετική προγνωστική τους αξία οφείλει να προσδιορίζεται από το Εργαστήριο για να κοινοποιηθεί στις Κλινικές.

Σκοπός: Η διερεύνηση ψευδώς θετικών αποτελεσμάτων κατά τον αρχικό έλεγχο διαλογής των δειγμάτων για τα παθογόνα Cytomegalovirus (CMV) και *Toxoplasma gondii* (TOXO).

Υλικό: Εξετάστηκαν 1.556 διαδοχικοί ασθενείς για CMV και 1.295 για TOXO που νοσηλεύτηκαν ή προσήλθαν στα εξωτερικά ιατρεία του νοσοκομείου «Αττικών» κατά την χρονική περίοδο από 01.01.12 έως 30.06.13.

Μέθοδος: Ως μέθοδος προσδιορισμού κατά τον αρχικό έλεγχο των IgG και IgM αντισωμάτων χρησιμοποιήθηκε ανοσο-χημειοφωταύγεια (αυτοματοποιημένος αναλυτής Architect, Abbott), ενώ για την επιβεβαίωση των θετικών IgM και IgG δειγμάτων και τον καθορισμό της IgG συνάφειας χρησιμοποιήθηκε η πρότυπη μέθοδος Enzyme Linked Fluorescent Assay (ELFA [αναλυτής VIDAS, Biomerieux]).

Αποτελέσματα: Α) Ως προς τον CMV, στο σύνολο 1.556 εξετασθέντων, 1.114 (71.5%) παρουσίασαν θετικά IgG και 108 (6.9%) θετικά IgM αντισώματα. Με τη μέθοδο ELFA επιβεβαιώθηκαν ως IgG θετικοί 78/87 (89.6%), και ως IgM 31/95 (32.6%). Μεταξύ 74 εξετασθέντων, 52 δείγματα ήταν υψηλής, 2 χαμηλής και 14 οριακής συνάφειας.

Β) Αναφορικά με το ΤΟΧΟ, στο σύνολο 1.295 εξετασθέντων, 256 (19.8%) παρουσίασαν θετικά IgG και 11 (0.9%) θετικά IgM αντισώματα. Από τους 11 τελευταίους, με τη μέθοδο ELFA επιβεβαιώθηκαν 10 (91%) ως IgG και 6 (54.6%) ως IgM θετικοί. Μεταξύ 10 εξετασθέντων, 7 δείγματα ήταν υψηλής, 2 χαμηλής και 1 οριακής συνάφειας.

Συμπεράσματα: Τα IgG θετικά αποτελέσματα αποτελέσματα του αναλυτή Architect για τα παθογόνα CMV και ΤΟΧΟ επιβεβαιώνονται από τη μέθοδο ELFA σε αρκετά υψηλό ποσοστό (89.6% και 91%), ενώ αντίθετα τα IgM θετικά σε ποσοστό μόλις 32.6% και 54.6%, αντίστοιχα. Η εφαρμογή της εξέτασης συνάφειας της IgG αναδεικνύει την πολύ χαμηλή συχνότητα οξέων λοιμώξεων στον πληθυσμό που εξετάζεται στο νοσοκομείο.

A (Μικροβιολογία) - 6

Συγκριτική μελέτη κρουσμάτων φυματίωσης στο Γ.Ν. Χανίων κατά τα έτη 2011-2012

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Εισαγωγή: Τα τελευταία έτη λόγω κοινωνικοοικονομικών αλλαγών, αύξησης του αριθμού των ανοσοκατασταλαμένων ατόμων και μετακίνησης πληθυσμών από ενδημικές χώρες, παρατηρείται αύξηση των λοιμώξεων από μυκοβακτηρίδια, η οποία αποτελεί σοβαρό πρόβλημα για τη δημόσια υγεία.

Σκοπός: Η καταγραφή της συχνότητας απομόνωσης μυκοβακτηριδίων από κλινικά δείγματα εσωτερικών και εξωτερικών ασθενών που προσήλθαν στο γενικό νοσοκομείο Χανίων κατά τα έτη 2011-2012 και η σύγκριση των αποτελεσμάτων.

Υλικά και μέθοδοι: Το υλικό αποτέλεσαν 648 κλινικά δείγματα που στάλθηκαν στο μικροβιολογικό τμήμα κατά τα έτη 2011-2012 από νοσηλευόμενους και εξωτερικούς ασθενείς. Στα δείγματα αυτά έγιναν άμεσα παρασκευάσματα για μικροσκοπηση με χρώση Ziehl-Nielsen και καλλιέργεια σε θρεπτικό υλικό Löwenstein-Jensen (bioMérieux). Οι θετικές καλλιέργειες στάλθηκαν στο Εθνικό Κέντρο Αναφοράς Μυκοβακτηριδίων, «Σωτηρία», όπου έγινε η τυποποίηση των μυκοβακτηριδίων.

Αποτελέσματα: Κατά το έτος 2011 από 283 καλλιέργειες κλινικών δειγμάτων για β-koch βρέθηκαν 8 θετικές (ποσοστό 2,1%). Όλα τα στελέχη τυποποιήθηκαν *M.tuberculosis* (ποσοστό 100%). Κατά το έτος 2012 από 365 καλλιέργειες κλινικών δειγμάτων για β-koch βρέθηκαν 11 θετικές (ποσοστό 3,01%). Τα στελέχη που τυποποιήθηκαν από τις 11 θετικές καλλιέργειες ήταν 9 *M.tuberculosis*, 1 *N.T.M.* και 1 *M.absessus immunogenum*.

Συμπεράσματα: Παρατηρήθηκε αύξηση των κρουσμάτων φυματίωσης το 2012 (3,01%) σε σχέση με το 2011 (2,1%). Όλα τα στελέχη που απομονώθηκαν και τυποποιήθηκαν το 2011 ήταν *M.tuberculosis* ενώ το 2012 απομονώθηκαν και τυποποιήθηκαν 9 στελέχη *M.tuberculosis* και 2 νέα στελέχη μυκοβακτηριδίου (*N.T.M.* και *M. absessus immunogenum*) που δεν είχαν εμφανιστεί το προηγούμενο έτος.

A (Μικροβιολογία) - 7

Legionella

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Εισαγωγή: Η Legionella εντοπίστηκε για πρώτη φορά το 1977 από το Κέντρο Πρόληψης και Ελέγχου ασθενειών (ECDC), μετά από επιδημία πνευμονίας που παρουσίασε μεγάλος αριθμός βετεράνων της Αμερικανικής Λεγεώνας, κατά την διάρκεια παρακολούθησης συνεδρίου τον Ιούλιο του 1976 στο ξενοδοχείο Bellevue Stratford στην Φιλαδέλφεια της Πενσυλβάνιας στις Η.Π.Α. Ονομάστηκε Legionella προς τιμήν των βετεράνων της Αμερικανικής Λεγεώνας που επλήγησαν. (Fraser et al, 1977; AWT 2003)

Σκοπός: της εργασίας αυτής είναι η περιγραφή του βακτηρίου της Λεγεωνέλλας, του νοσήματος που προκαλεί, των μεθόδων διάγνυσής της, του τρόπου μετάδοσής της, καθώς επίσης και των μέτρων προφύλαξης.

Υλικά μέθοδοι: Η Legionella pneumophila είναι ένα Gram- αρνητικό βακτήριο, λεπτό, που βρίσκεται παντού στο υδάτινο περιβάλλον και αναπτύσσεται σε θερμοκρασίες 20°C-40°C. (Γενική Μικροβιολογία, Τρίτη έκδοση, εκδόσεις Έλλην). Οι μικροοργανισμοί του γένους των λεγεωνελλών είναι διαδεδομένοι τόσο στο φυσικό όσο και στο τεχνητό περιβάλλον του ανθρώπου. Απομονώνονται από το νερό ποταμών, ρυακίων, λιμνών, καθώς επίσης και από το χώμα Η σοβαρότητα της νόσου που προκαλεί ποικίλει από μια ήπια εμπύρετη ασθένεια, πυρετός Pontiac, μέχρι σοβαρής μορφής πνευμονία, νόσος των Λεγεωνάριων. Μεταδίδεται αερογενώς μέσω των εισπνεόμενων σταγονιδίων, ενώ μέχρι σήμερα δεν έχει διαπιστωθεί μετάδοση της νόσου από άτομο σε άτομο. Αναπτύσσεται εύκολα σε συστήματα ζεστού και κρύου νερού σε κτίρια (νοσοκομεία, ξενοδοχεία), σε νερό πύργων ψύξης των συστημάτων κλιματισμού, σε δεξαμενές αποθήκευσης νερού, κολυμβητικές δεξαμενές, πισίνες, σιντριβάνια, υγραντήρες, αναπνευστικές συσκευές, ντουςιέρες και θερμοσίφωνα κ.α. (WHO, Water Recreation and Disease, 2005). Η εργαστηριακή διάγνωση γίνεται σε κλινικά δείγματα αλλά και σε περιβαλλοντικά δείγματα. 1986 συγκροτήθηκε η Ευρωπαϊκή Ομάδα Εργασίας για την Νόσο των Λεγεωνάριων EWGLI: (European Working Group for Legionella Infections) και το 1987 υλοποιήθηκε η επιτήρηση των περιπτώσεων της Νόσου των Λεγεωνάριων που συνδέονται με ταξίδια, μέσω του Ευρωπαϊκού Δικτύου Επιτήρησης της Νόσου των Λεγεωνάριων. Στην Ελλάδα, η Νόσος των Λεγεωνάριων αποτελεί νόσημα υποχρεωτικής δήλωσης σε χρονικό διάστημα 24 ωρών από την διάγνωση του. (Υπουργείο Υγείας, Γενική Δ/ση Δημόσιας Υγείας & Ποιότητας Ζωής, "Πρόληψη νόσου των λεγεωναρίων", Αθήνα 2012). Για την αποφυγή της ανάπτυξης των βακτηριδίων θα πρέπει να λαμβάνονται προληπτικά μέτρα: Να γίνεται σχολαστική έρευνα για την εκτίμηση κινδύνου που συμπεριλαμβάνει τον έλεγχο όλων των συστημάτων νερού και των εγκαταστάσεων κλιματισμού. Καλή συντήρηση των συστημάτων ψύξης και των υδραυλικών συστημάτων. Κατάλληλο προσωπικό για την συντήρηση των υδραυλικών συστημάτων και των εγκαταστάσεων κλιματισμού Διατήρηση του ζεστού νερού πάνω από 60°C Η θεραπεία βασίζεται στην έγκαιρη χορήγηση των αντιβιοτικών που είναι αποτελεσματικά κατά της λεγιονέλλας.

Αποτελέσματα: Το βακτήριο Legionella προκαλεί τη νόσο των λεγεωναρίων και τον πυρετό Pontiac. Οι κύριες πηγές μόλυνσης είναι οι εγκαταστάσεις κλιματισμού και τα λιμνάζοντα νερά. Για την αποφυγή της ανάπτυξης των βακτηριδίων θα πρέπει να λαμβάνονται προληπτικά μέτρα.

A (Μικροβιολογία) - 8

Επιδημιολογική διερεύνηση της συχνότητας, της εποχιακής και ηλικιακής κατανομής των στρεπτόκοκκων σε παιδιατρικά νοσοκομεία της Αττικής κατά τη τριετία 2008-2010

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Νοσοκομείο Παιδων Αγία Σοφία

Εισαγωγή: Τα στελέχη στρεπτοκόκκων είναι σε μεγάλο βαθμό μολυσματικά και μεταδίδονται πολύ εύκολα είτε με άμεση επαφή είτε μέσω εκκρίσεων του αναπνευστικού συστήματος. Προκαλούν ένα ευρύ φάσμα νοσημάτων στον άνθρωπο και είναι εδώ και αιώνες. Τα κλινικά σύνδρομα από τον β αιμολυτικό στρεπτόκοκκο της ομάδας Α είναι η φαρυγγίτιδα, η αμυγδαλίτιδα, η οστρακιά, οι φλεγμονές του δέρματος, οι λοιμώξεις σε βαθύτερους ιστούς, τα αυτοάνοσα νοσήματα. Ο πνευμονιόκοκκος είναι ένα

βακτήριο που προκαλεί σοβαρή ασθένεια, συχνά απειλητική για τη ζωή των μικρών παιδιών. Ο Παγκόσμιος Οργανισμός Υγείας εκτιμά ότι 1 εκατομμύριο παιδιά κάτω των πέντε ετών, χάνουν τη ζωή τους κάθε χρόνο από πνευμονιοκοκκική νόσο.

Σκοπός: Η παρούσα έρευνα έχει σαν αντικείμενο τη μελέτη των στρεπτοκόκκων στον παιδιατρικό πληθυσμό (0 έως 15 ετών), σε δύο κύρια παιδιατρικά νοσοκομεία της Αττικής: Νοσοκομείο Παιδων 'Αγία Σοφία' και Νοσοκομείο Παιδων Πεντέλης

Σκοπός της έρευνας αυτής είναι:

1. Η καταγραφή της συχνότητας των περιστατικών από τα *streptococcus spp.* ανάλογα με το μέρος απομόνωσης τους (π.χ. φάρυγγας, εγκεφαλονωτιαίου υγρού).
2. Η εποχιακή κατανομή των περιστατικών.
3. η ηλικιακή κατανομή των περιστατικών.
4. Ο έλεγχος της αντοχής των στελεχών των στρεπτοκόκκων στα αντιβιοτικά.

Υλικό: Μελετήθηκαν οι καταγραφές από τα αρχεία των νοσοκομείων παιδων 'Αγία Σοφία' (νοσοκομείο Α) και παιδων 'Πεντέλης' (νοσοκομείο Β) κατά την τριετία 2008-2010 ως προς:

1. Τις καλλιέργειες φαρυγγικών επιχρισμάτων
2. Τη δοκιμασία ταχείας διάγνωσης για την τυποποίηση του μικροοργανισμού *Streptococcus pyogenes* ομάδας Α (strep test)
3. Τις καλλιέργειες εγκεφαλονωτιαίου υγρού (ENY).
4. Συνολικά κατά την τριετία 2008-2010, πραγματοποιήθηκαν 11.281 καλλιέργειες φαρυγγικού επιχρίσματος (6009 στο νοσοκομείο Α) και 5272 στο νοσοκομείο Β), εκ των οποίων 755 (12,6%) από το νοσοκομείο Α και 1346 (25,5%) από το νοσοκομείο Β βρέθηκαν θετικές για κάποιο παθογόνο μικροοργανισμό, ο οποίος ταυτοποιήθηκε και ελέγχθηκε ως προς την αντοχή του στα αντιβιοτικά. Στο νοσοκομείο Α πραγματοποιήθηκαν: 20939 δοκιμασίες ταχείας διάγνωσης για την τυποποίηση του GAS και στο νοσοκομείο Β: 16.675. Οι καλλιέργειες εγκεφαλονωτιαίου υγρού ανέρχονται στις 4206 για το νοσοκομείο Α και στις 336 για το νοσοκομείο Β εκ των οποίων οι 233 (5,5%) και 11(3,2%) αντίστοιχα, ανέπτυξαν κάποιο παθογόνο μικροοργανισμό . Ακολούθησε ταυτοποίηση και αντιβιογράμμα.

Μεθοδολογία:

1. Καλλιέργεια φαρυγγικού επιχρίσματος.
2. Δοκιμασία ταχείας διάγνωσης για την τυποποίηση του μικροοργανισμού *Streptococcus pyogenes* ομάδας Α (strep test).
3. Καλλιέργεια εγκεφαλονωτιαίου υγρού.
4. Στατιστική επεξεργασία.

Αποτελέσματα – Συμπεράσματα: Κατά την παραπάνω χρονική περίοδο πραγματοποιήθηκαν 11.281 καλλιέργειες φαρυγγικού επιχρίσματος, στα νοσοκομεία παιδων 'Αγία Σοφία' και 'Πεντέλη'. 2101 καλλιέργειες (18,1%) βρέθηκαν θετικές για παθογόνους μικροοργανισμούς. Από αυτές ένα μεγάλο ποσοστό (94,4%, 1981/2101), βρέθηκαν θετικές για β αιμολυτικό στρεπτόκοκκο ομάδας Α, ενώ 38 (1,8%) βρέθηκαν θετικές για β αιμολυτικό στρεπτόκοκκο ομάδας C και 38 (1,8%) ομάδας G. Κατά το έτος 2009 (έτος που εμφανίστηκε για πρώτη φορά ο ιός H1N1) οι θετικές καλλιέργειες φαρυγγικού επιχρίσματος ανέρχονται στις 962 (46% των θετικών καλλιεργειών της τριετίας). Οι αιτίες παραπομπής σε καλλιέργεια είναι εμπύρετο περιστατικό (44,7%) και φαρυγγαλγία (17,5%). Ο *S. pyogenes* έχει εποχιακή διακύμανση με την άνοιξη να είναι η εποχή με τις περισσότερες θετικές καλλιέργειες (707 καλλιέργειες την τριετία 2008-2010 ~ 36%) και το φθινόπωρο την εποχή με τις λιγότερες (341 καλλιέργειες την τριετία 2008-2010: ~17%). Από τα 1981 αντιβιογράμματα που πραγματοποιήθηκαν, βρέθηκε πως ο β αιμολυτικός στρεπτόκοκκος ομάδας Α είναι ανθεκτικός στα αντιβιοτικά: ερυθρομυκίνη (19,7%) κλινταμικίνη (17,1%). Η αντοχή αυτή βρέθηκε πως είναι επαγώγιμη από την ερυθρομυκίνη στο 80% των περιπτώσεων (D- test). Από τις 37.614 δοκιμασίες ταχείας διάγνωσης που πραγματοποιήθηκαν 7084 βρέθηκαν θετικές για *S. Pyogenes*. Τα αγόρια και τα κορίτσια νοσούν με την ίδια συχνότητα. Οι ηλικίες με τις περισσότερες θετικές καλλιέργειες φαρυγγικού επιχρίσματος και δοκιμασίες ταχείας διάγνωσης ήταν στην ηλικιακή ομάδα 5-9 ετών (p=0,001). Τέλος, πραγματοποιήθηκαν 4542 καλλιέργειες ENY εκ των οποίων 13 βρέθηκαν θετικές για *Streptococcus pneumoniae*. Από τα αντιβιογράμματα προκύπτει ο *Streptococcus pneumoniae* είναι ανθεκτικός στην πενικιλίνη (25%) στην ερυθρομυκίνη

(33%) στην οξακυκλίνη (17%) και στην κοτριμοξαζόλη (17%). Κατά το 2009, υπήρξε αύξηση των καλλιιεργειών ENY, που βρέθηκαν θετικές για πνευμονιόκοκκο (46% των θετικών καλλιιεργειών της τριετίας), ($p=0,02$).

A (Μικροβιολογία) - 9

Η εκπαίδευση και η ενημέρωση του προσωπικού νοσοκομείων αστικού συγκροτήματος μεσαίας δυναμικότητας

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Εισαγωγή. Θεμελιώδης κρίνεται η συνεχής εκπαίδευση του υγειονομικού προσωπικού, ώστε να είναι σε θέση να αναγνωρίζει και να προβλέπει τους πιθανούς κινδύνους, να ακολουθεί τις βασικές αρχές πρόληψης των επαγγελματικών ατυχημάτων στον χώρο εργασίας, αλλά και να χρησιμοποιεί σωστά τα κατάλληλα μέσα ατομικής προστασίας, που προβλέπονται κατά περίπτωση (Λινού Α., Ιατρική της Εργασίας, Εκδόσεις Βήτα, (c) 2005-Βελονάκης Μ., Τσαλίκουλου Φ., Συστήματα διαχείρισης υγείας και ασφάλειας κατά της εργασίας σε νοσοκομείο. Εκδόσεις Παρισιάνου, 2005 - Παπαδόπουλος Γ., Καλοβούλου Λ., Σοφός Α., Νοσοκομειακές λοιμώξεις, Επιδημιολογία, Πρόληψη, Έλεγχος, Εκδόσεις Παρισιάνου, 1997).

Σκοπός. Να επισημανθεί η πιθανή προτεραιότητα του Κράτους, ως εργοδότη, στην διαδικασία της συνεχούς επιμόρφωσης του υγειονομικού προσωπικού του.

Υλικά και μέθοδος. Χρησιμοποιήθηκε ελληνική ιατρική βιβλιογραφία και ερωτηματολόγιο 152 ερωτήσεων, που μοιράστηκε στο σύνολο των εργαζομένων του 1^{ου} Γ.Π.Ν.Θ «Αγ.Παύλος». Διήρκεσε η διαδικασία 3 μήνες (1/4/2010-30/6/2010) και απαντήθηκαν 480 ερωτηματολόγια. Τα αποτελέσματα μελετήθηκαν ως προς τέσσερις εργασιακούς χώρους: διοικητικές υπηρεσίες, εργαστήρια, κλινικές και μονάδες.

Αποτελέσματα. Μετρήθηκε πως επαρκή ενημέρωση για τους κινδύνους, που ελλοχεύουν στο χώρο εργασίας, έχουν μόνο το 30,4% του προσωπικού, το 24% δηλώνει πως στερείται ενημέρωσης και το 45,6% πληροφορείται μερικώς. Η καλύτερη ενημέρωση καταγράφεται στις Κλινικές με 41,2% και η χειρότερη στα Εργαστήρια 27,9%. Η ολοκληρωμένη εκπαίδευση στην πρόληψη και στην αντιμετώπιση των κινδύνων φθάνει μόλις το 15,2% και 15,8% αντίστοιχα, ενώ η απουσία κατάρτισης για τα παραπάνω αγγίζει το 38% και το 38,6% ομοίως. Τονίζεται ειδικότερα ότι στους Διοικητικούς η μη εκπαίδευση στην πρόληψη ακουμπά το 44,4% και στους Κλινικούς η απουσία από την έγκαιρη εκπαίδευση στην αντιμετώπιση προσεγγίζει το 44,1%. Όσον αφορά την συμμετοχή του προσωπικού σε εισηγήσεις ημερίδων-συνεδρίων δεν ξεπερνά το 15,2% επί του συνόλου, με το ποσοστό αυτό να μηδενίζεται στο Διοικητικό Προσωπικό και στα Εργαστήρια να αγγίζει το 21,3%. Στην παρακολούθηση και ακρόαση ημερίδων-συνεδρίων ανταποκρίνεται το 49,4% και απουσιάζει εντελώς το 17,7%. Ειδικότερα οι Εργαστηριακοί που παρακολουθούν τέτοιες δραστηριότητες είναι το 70,5% και απέχουν το 6,5%. Τέλος στη λήψη συγκεκριμένων μέτρων προστασίας από τους κινδύνους προχωρά το 50,6% του Προσωπικού, που στα Εργαστήρια φθάνει στο 54,1% . Αδιαφορεί παντελώς για τα μέτρα προστασίας το 24%, που στις Κλινικές προσεγγίζει το 26,5%.

Συμπεράσματα. Το κράτος ως εργοδότης υστερεί σημαντικότερα στην επαρκή ενημέρωση των υγειονομικών στελεχών και υπαλλήλων του, παρότι τα νοσηλευτικά ιδρύματα χαρακτηρίζονται ζώνες υψηλού κινδύνου για λοιμογόνους παράγοντες. Δεν δίνει επίσης κίνητρα για έρευνα και μεταπτυχιακή κατάρτιση. Το προσωπικό ωστόσο, επιδεικνύοντας υψηλό αίσθημα ευθύνης, αυτοβελτιώνεται σημαντικά δια της παρακολούθησης ημερίδων-συνεδρίων και λαμβάνει μέτρα στην αντιμετώπιση των εργασιακών κινδύνων για τους ίδιους και για τους ασθενείς.

A (Μικροβιολογία) - 10

Απολυμαντικά – αντισηπτικά είδη, τρόπος δράσης και ο ρόλος τους στην ανάπτυξη της μικροβιακής αντοχής

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Εισαγωγή. Όπως προκύπτει από τη μελέτη των ιστορικών κειμένων η απολύμανση ήταν γνωστή ως εμπειρική μέθοδος προστασίας του ανθρώπου από τις λοιμώξεις αιώνες πριν από την εδραίωση της μικροβιακής θεωρίας των νόσων από τους Louis Pasteur και Robert Koch.

Σκοπός της εργασίας είναι η προσέγγιση της χρήσης των απολυμαντικών και των αντισηπτικών στο χώρο της υγείας ο τρόπος δράσης τους καθώς και η συσχέτιση τους με την ανάπτυξη μικροβιακής αντοχής. Τα απολυμαντικά και τα αντισηπτικά χρησιμοποιούνται ευρέως στο χώρο της υγείας. Απολύμανση ορίζεται η εξάλειψη ή μείωση >3log cfu του αριθμού των παθογόνων μικροοργανισμών εκτός των σπόρων από αντικείμενα ή επιφάνειες ώστε να μην μπορούν να προκαλέσουν λοίμωξη. Ως αντισηπτικά χρησιμοποιούνται κυρίως οι αλκοόλες, η χλωρεξιδίνη, το ιώδιο, και τα ινωδοφόρα, η χλωροξυλενόλη και η τρικλοζάνη. Η αποτελεσματικότητα της δράσης των απολυμαντικών κατά των μικροοργανισμών εξαρτάται από πολλούς παράγοντες. Η χρήση των αντισηπτικών αφορά κυρίως την αντισηψία χεριών του ιατρονοσηλευτικού και παραϊατρικού προσωπικού, την προεγχειρητική αντισηψία δέρματος των ασθενών, την προετοιμασία δέρματος πριν από την εφαρμογή καθετηριασμών και την περιποίηση τραυμάτων. Ο μηχανισμός δράσης των απολυμαντικών-αντισηπτικών διαφοροποιείται ανάλογα με τη χημική δομή τους. Ο βιολογικός στόχος δράσης μπορεί να είναι το κυτταρικό τοίχωμα, η κυτταροπλασματική μεμβράνη, το γενετικό υλικό, οι μακρομοριακές ουσίες του κυτταροπλάσματος κ.α. Μεταξύ των βακτηρίων που απομονώνονται στο νοσοκομειακό περιβάλλον οι σταφυλόκοκκοι και οι εντερόκοκκοι είναι σχετικά πιο ανθεκτικοί από άλλα Gram θετικά βακτήρια. Τα Gram αρνητικά βακτήρια όπως αυτά που ανήκουν στα γένη *Pseudomonas*, *Klebsiella*, *Enterobacter* και *Serratia* θεωρούνται σχετικά ανθεκτικά στη δράση ορισμένων απολυμαντικών-αντισηπτικών.

Συμπέρασμα. Στην εποχή μας η ανάπτυξη μικροβιακής αντοχής αποτελεί ένα από τα μεγαλύτερα προβλήματα που απασχολούν τη διεθνή κοινότητα στον τομέα έλεγχου λοιμώξεων. Συνεπακόλουθα υπάρχουν προβληματισμοί που αφορούν τη μικροβιακή αντοχή και τη χρήση απολυμαντικών-αντισηπτικών στο χώρο της υγείας. Δεν θα πρέπει να ξεχνάμε ότι η χρησιμοποίηση των απολυμαντικών δεν θα πρέπει να μας δίνει την ψευδαίσθηση της ασφάλειας και το αίσθημα της σιγουριάς.

A (Μοριακή βιολογία) – 1

Ο ρόλος των γονιδίων στην αιτιοπαθογένεια της παχυσαρκίας

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Εισαγωγή: Σε παγκόσμιο επίπεδο, η πρόοδος στις γνώσεις μας σχετικά με το ρόλο των γονιδίων στην αιτιοπαθογένεια της παχυσαρκίας, υπήρξε αξιοσημείωτη τα τελευταία χρόνια.

Σκοπός: Σκοπός της συγκεκριμένης εργασίας, είναι η βιβλιογραφική ανασκόπηση των γενετικών εκείνων παραγόντων και των μεταλλάξεων που εμπλέκονται στην εκδήλωση της πολυπαραγοντικής κλινικής νόσου της παχυσαρκίας, που αποτελεί ένα παγκόσμιο πρόβλημα της δημόσιας υγείας.

Υλικό: Εργαλεία αναζήτησής μας, αποτέλεσαν οι διεθνείς βάσεις επιστημονικών άρθρων Pubmed, Scopus και η ελληνική ηλεκτρονική πύλη ιατρικών δεδομένων: <http://panacea.med.uoa.gr>, αλλά και διάφορα ελληνικά επιστημονικά συγγράμματα σχετικά με το ρόλο της γενετικής στην αιτιοπαθογένεια της παχυσαρκίας.

Αποτελέσματα: Το 2001, μόνο έξι γονίδια είχαν συνδεθεί με την πρόκληση μονογονιδιακών μορφών παχυσαρκίας, ενώ κανένας γενετικός πολυμορφισμός δεν είχε σχετιστεί επανειλημμένα με πολυγονιδιακές μορφές. Το 2007 και έπειτα, η εφαρμογή των μελετών σάρωσης ολόκληρου του γονιδιώματος (GWAs, Genome-Wide Association studies) και η αυτοματοποίηση των τεχνολογιών ανάλυσης της αλληλουχίας του DNA έφερε επανάσταση στη γενετική της παχυσαρκίας. Σήμερα είναι γνωστό, ότι 8 γονίδια και το έλλειμμα 16p11.2 εμπλέκονται σε μεντελικές μονογονιδιακές μορφές παχυσαρκίας και επίσης 61 μεμονωμένοι νουκλεοτιδικοί πολυμορφισμοί (SNPs) σε 58 γονίδια, σχετίζονται ισχυρά (σε τιμές $P < 5 \times 10^{-8}$) με την πολυγονιδιακή παχυσαρκία (Choquet H, Meyre D. 2011). Οι μονογονιδιακές ή μεντελικές μορφές παχυσαρκίας, συνιστούν το 5-10% των περιπτώσεων παχυσαρκίας και αποδίδονται κυρίως στην παρουσία μεταλλάξεων σε μεμονωμένα γονίδια που δρουν στο μονοπάτι λεπτίνης- μελανοκορτίνης του υποθαλάμου. **Η λεπτίνη**, το παράγωγο του ob (-obese) γονιδίου της παχυσαρκίας, είναι ένα 16 KDa μη γλυκοζυλιωμένο πολυπεπτίδιο, που αποτελείται από 146 αμινοξέα και ανακαλύφθηκε το 1994 από τον Zhang και τους συνεργάτες του (Zhang et al. 1994). Μεταλλάξεις στο γονίδιο που κωδικοποιεί τη λεπτίνη (ob), έχουν ως αποτέλεσμα τα γενετικά παχύσαρκα ποντίκια. Τα ob/ob μεταλλαγμένα ποντίκια παράγουν μια ελαττωματική, μη λειτουργική λεπτίνη, καταλήγοντας σε ένα φαινότυπο παχύσαρκο και υπογόνιμο, επειδή η λεπτίνη δεν μπορεί να μεταφέρει το σήμα στον εγκέφαλο (Tartaglia et al. 1997). Μελέτες σε επίπεδο μεταλλάξεων και πολυμορφισμών SNPs, έχουν καθιερώσει το γονίδιο MC4R, ως ένα από τα σημαντικότερα γονίδια σχετικά με την παχυσαρκία. Έχουν περιγραφεί πάνω από 150 σημειακές μεταλλάξεις, οι οποίες οδηγούν σε απώλεια λειτουργίας του υποδοχέα (Tao YX. 2010). Στις πολυγονιδιακές μορφές, το γονίδιο FTO, με περισσότερα από 400 δημοσιευμένα άρθρα, θεωρείται μέχρι σήμερα το πιο ισχυρά σχετιζόμενο με την παχυσαρκία, γονίδιο. Εδράζεται στο χρωμόσωμα 16.

Συμπεράσματα: Η προδιάθεση για παχυσαρκία είναι μερικώς προκαθορισμένη από το γενετικό υπόβαθρο, ωστόσο η ύπαρξη «παχύσαρκου» περιβάλλοντος, είναι απαραίτητη για τη φαινοτυπική έκφραση της παχυσαρκίας. Παραμένει ακόμα να διευκρινιστεί ο σωστός συνδυασμός των γονιδίων και των μεταλλάξεων και να ανακαλύψουμε με ποιον τρόπο, οι περιβαλλοντικοί παράγοντες αλληλεπιδρούν στην τελική διαμόρφωση του κινδύνου εκδήλωσης παχυσαρκίας. Η διερεύνηση της συνεισφοράς των γονιδίων στην εκδήλωση παχυσαρκίας προσφέρει σημαντικά πλεονεκτήματα, γιατί θα μπορεί μελλοντικά να συμβάλει: α) στην κατανόηση της φυσιοπαθολογίας και των μηχανισμών πρόκλησης παχυσαρκίας, β) στην ταυτοποίηση ατόμων που βρίσκονται σε υψηλό κίνδυνο εμφάνισης της νόσου, και γ) στην εξατομικευμένη ιατρική αντιμετώπιση των παχύσαρκων ατόμων.

A (Μοριακή βιολογία) – 2

Πρόγνωση και διάγνωση της δυσλεξίας

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Εισαγωγή: Η δυνατότητα ανάγνωσης, γραφής, κατανόησης και χρήσης της γλώσσας αποτελεί απαραίτητο στοιχείο για την κοινωνική ενσωμάτωση. Η δυσχέρεια στην απόκτηση αυτών των ικανοτήτων καλείται δυσλεξία, μία δυσλειτουργία της οποίας η αιτιολογία δεν έχει αποδοθεί με ακρίβεια, λόγω της πολυπλοκότητάς της και απασχολεί την επιστημονική κοινότητα τα τελευταία χρόνια.

Σκοπός: Σκοπός της συγκεκριμένης εργασίας είναι η εύρεση μεθόδων έγκαιρης πρόγνωσης και διάγνωσης της δυσλεξίας με ανασκόπηση της βιβλιογραφίας.

Μέθοδος ανασκόπησης: Έγινε αναζήτηση της βιβλιογραφίας στις βάσεις δεδομένων PubMed και Google Scholar, με τη χρήση των ακόλουθων λέξεων κλειδών: dyslexia genes- dyslexia genetics, f-MRI dyslexia genes, imaging methods of dyslexia, automated prognosis tools, learning difficulties, machine learning.

Αποτελέσματα: Η έγκαιρη πρόγνωση μπορεί να γίνει με απεικονιστικές μεθόδους (PET, f-MRI, DTI, MEG, EEG) οι οποίες ανιχνεύουν τη διαφορά στη λειτουργικότητα των περιοχών του εγκεφάλου μεταξύ δυσλεκτικών και μη. Πιο συγκεκριμένα, από παθολογοανατομικής - παθοφυσιολογικής πλευράς το πρόβλημα των δυσλεκτικών έγκειται στο αριστερό ημισφαίριο στην ινιακοβρεγματική και στην κροταφοβρεγματική αύλακα. Παρουσιάζουν προβλήματα όπως η ελλιπής ημισφαιρική κυριαρχία, που

αφορά το κέντρο των γλωσσικών λειτουργιών. Αυτή προκαλείται συνήθως από υστέρηση μετανάστευσης των νευρώνων κατά την ανάπτυξη του εγκεφαλικού φλοιού στα βαθύτερα στρώματα του νεοφλοιού ή μετανάστευση σε λάθος περιοχή κατά την δημιουργία νευρωνικών δικτύων. Η διάγνωση μπορεί επίσης να γίνει με τη χρήση εξειδικευμένων προγραμμάτων H/Y (MAPS, WISC I, II, III, Wimmer, Mastery Woodcock) με τα οποία μπορούμε όχι μόνο να διαγνώσουμε τη σοβαρότητα της δυσλεξίας, αλλά και π.χ. το είδος αυτής, όπως φωνολογική, ακουστική και οπτική.

Επιπλέον, στην πρόγνωση και τη διάγνωση βοηθάει ο συσχετισμός των γενετικών δεικτών για τη δυσλεξία, όπως ROBO1, KIAA0319, DCDC2, με παθολογοανατομικά ευρήματα από τη μέθοδο f-MRI.

Συμπεράσματα: Με τις απεικονιστικές μεθόδους εντοπίζονται παθολογοανατομικά ευρήματα των περιοχών του εγκεφάλου, που μπορεί να οδηγήσουν σε πρόγνωση της δυσλεξίας. Η πρόγνωση αυτή ενισχύεται και με τη χρήση γενετικών δεικτών. Επιπλέον, η χρήση προγραμμάτων H/Y, λειτουργεί ως εργαλείο εντοπισμού της σοβαρότητας και του είδους της δυσλεξίας, με ποσοστό ταξινόμησης μεγαλύτερο της τάξεως του 90%.

A (Μοριακή βιολογία) – 3

Συχνότητα της μετάλλαξης G1691A του γονιδίου του παράγοντα V της πήξης του αίματος (V-LEIDEN) σε νεαρά άτομα με ιστορικό συγγενούς θρομβοφιλίας (πρόδρομη μελέτη)

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Εισαγωγή: Η θρομβοφιλία αποτελεί σημαντικό ιατρικό πρόβλημα, το οποίο επηρεάζει περίπου 1 στα 1000 άτομα ανά έτος. Η κληρονομική θρομβοφιλία αποτελεί μια γενετικά καθορισμένη τάση ανάπτυξης φλεβικής θρομβοεμβολής, η οποία είναι συχνά επαναλαμβανομένη. Ο παράγοντας V Leiden είναι η πιο κοινή μορφή κληρονομικής θρομβοφιλίας, αντιπροσωπεύοντας το 40-50% των περιπτώσεων. Η θρομβοφιλία V Leiden είναι μια γενετική διαταραχή που χαρακτηρίζεται από μια ασθενή αντιθρομβωτική απόκριση στην ενεργοποιημένη πρωτεΐνη C (APC) και αυξημένο κίνδυνο εμφάνισης φλεβικής θρομβοεμβολής. Σε μοριακό επίπεδο, η FV θρομβοφιλία συσχετίζεται κυρίως με μια νουκλεοτιδική αντικατάσταση (G1691A) η οποία έχει ως αποτέλεσμα μια αμινοξική αντικατάσταση (Arg506Gln) στην APC.

Σκοπός: Οι FV ομοζυγώτες εμφανίζουν μεγαλύτερο κίνδυνο θρόμβωσης και συνήθως την εκδηλώνουν σε μικρή ηλικία (μεταξύ 25 και 35 ετών). Σκοπός της παρούσας μελέτης ήταν η διερεύνηση της συχνότητας της μετάλλαξης FV G1691A του γονιδίου του παράγοντα V της πήξης του αίματος (παράγοντας V-Leiden, FVL) σε νεαρά άτομα με ιστορικό συγγενούς θρομβοφιλίας

Υλικό και Μέθοδοι: Στη μελέτη συμπεριλήφθηκαν 42 νεαρά άτομα με ιστορικό συγγενούς θρομβοφιλίας το οποίο διερευνήθηκε με ερωτηματολόγιο για τρεις γενιές. Το DNA των υπό μελέτη ατόμων απομονώθηκε από δείγματα περιφερικού αίματος. Στη συνέχεια εφαρμόστηκε αλυσιδωτή αντίδραση πολυμεράσης (PCR) ακολουθούμενη από υδρόλυση με το ένζυμο περιορισμού *MnlI* και ηλεκτροφόρηση σε πηκτική αгарόζη. Η λήψη των δειγμάτων και η διανομή των ερωτηματολογίων διενεργήθηκε στο Εργαστήριο Αιματολογίας.

Αποτελέσματα: Από τα 42 δείγματα που μελετήσαμε, τα 7 ήταν ετερόζυγα GA (16.7 %) και τα υπόλοιπα 35 (83.3 %) ήταν ομόζυγα για το φυσιολογικό αλληλόμορφο (GG). Δείγματα ομόζυγα για την μετάλλαξη G1691A δε βρέθηκαν στον υπό μελέτη πληθυσμό της παρούσας εργασίας. Οι συχνότητες των αλληλίων ήταν 0.92 και 0.08 για το G και το A αλληλίο αντίστοιχα.

Συμπεράσματα: Ετεροζυγωτία για τον FVL εμφανίζεται στο 3-8 % του γενικού πληθυσμού σχεδόν παγκόσμια, ενώ η συχνότητα της ομοζυγωτίας στους λευκούς πληθυσμούς είναι περίπου 1/5000. Στην Ελλάδα, η συχνότητα της ετεροζυγωτίας στο γενικό πληθυσμό ανέρχεται έως και το 15%, ενώ ιδιαίτερα στη Β. Ελλάδα εμφανίζεται συχνότητα της τάξης του 4.8 %. Παρά το γεγονός ότι το δείγμα της μελέτης μας είναι μικρό, η συχνότητα της μετάλλαξης FV Leiden εμφανίζεται σχετικά αυξημένη σε άτομα με ιστορικό συγγενούς θρομβοφιλίας, συγκριτικά με το γενικό πληθυσμό και ιδιαίτερα της Β. Ελλάδας.

A (Παθολογική Ανατομική) - 1

Νόσος του Crohn στην παιδική ηλικία

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Γ.Ν.Θ. Παπαγεωργίου

Σκοπός-Εισαγωγή: Είναι ιδιοπαθής φλεγμονώδης νόσος του εντέρου και διαγιγνώσκεται σε ποσοστό 20-30% σε παιδιά και εφήβους. Τα τελευταία χρόνια παρατηρείται αύξηση της επίπτωσης της νόσου του Crohn στα παιδιά, ενώ η επίπτωση της ελκώδους κολίτιδας παραμένει στα ίδια επίπεδα. Η συμπτωματολογία της νόσου στα παιδιά είναι ίδια με αυτή των ενηλίκων. Συχνά παρατηρείται καθυστέρηση της ανάπτυξης, αλλά και της έναρξης της εφηβείας. Σε ποσοστό περίπου 50% η νόσος εντοπίζεται στον τελικό ειλεό και στο εγγύς κόλον.

Υλικό και Μέθοδος : Από το αρχείο του παθολογοανατομικού εργαστηρίου του Γ.Ν.Θ ΠΑΠΑΓΕΩΡΓΙΟΥ βρέθηκαν 7 περιπτώσεις νόσου του Crohn σε παιδιά την τελευταία διετία. Οι 4 περιπτώσεις αφορούν άρρενες και οι 3 θήλεα. Η έναρξη της νόσου εντοπίζεται στην δεύτερη δεκαετία της ζωής και το ηλικιακό φάσμα της εργασίας μας είναι από 12 έως 16 ετών, με Μ.Ο ηλικίας τα 13 έτη. Σύμφωνα και με τα αναφερόμενα στη βιβλιογραφία, η νόσος προσβάλλει συχνότερα τον τελικό ειλεό και τμήμα η τμήματα του παχέος εντέρου.

Αποτελέσματα - Συμπεράσματα: Η διάγνωση βασίζεται στην κλινική εικόνα, τα ακτοσκοπικά, ενδοσκοπικά και ιστολογικά ευρήματα. Σε ποσοστό 20 – 30 % της ΙΦΝΕ στα παιδιά, οι αλλοιώσεις στο παχύ έντερο είναι δύσκολο να διακριθούν ως ελκώδης κολίτιδα, ή νόσος του Crohn. Υπάρχουν όμως αρκετές διαφορές στη συμπτωματολογία ανάμεσα στα παιδιά και στους ενήλικες. Έτσι, στα παιδιά είναι συχνότερη η προσβολή στο ανώτερο πεπτικό σύστημα και κυρίως στο παχύ έντερο. Όσον αφορά τα ιστολογικά χαρακτηριστικά, στα παιδιά είναι συχνότερα τα κοκκιώματα, ενώ η φλεγμονή έχει σε μικρότερα ποσοστά από τους ενήλικες εστιακό χαρακτήρα

A (Παθολογική Ανατομική) - 2

Οικογενείς αδενωματώδης πολυποδίαση

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Είναι μια σπάνια κληρονομική νόσος που χαρακτηρίζεται από την ανάπτυξη μεγάλου αριθμού πολυποδών στο παχύ έντερο. Και τα δύο φύλα προσβάλλονται το ίδιο, ενώ η επίπτωση της πάθησης στο γενικό πληθυσμό είναι 1/8000γεννήσεις. Συνήθως αναγνωρίζονται περισσότεροι από 100 πολύποδες, ενώ, αναφορικά με το μέγεθός τους, μόνο το 1% είναι μεγαλύτεροι από 1 εκατοστό και έχουν ιστολογικά χαρακτηριστικά τυπικών αδενωμάτων. Εκτός από το παχύ έντερο, οι πολύποδες του συνδρόμου, μπορεί να εντοπίζονται στον στόμαχο, στο δωδεκαδάκτυλο, στον χοληδόχο πόρο στο φύμα του Vater, καθώς και στην νήστιδα και τον τελικό ειλεό.

Οι ασθενείς με οικογενή πολυποδίαση εμφανίζουν μετάλλαξη του γονιδίου APC, που εντοπίζεται στο μακρύ σκέλος του χρωμοσώματος 5 και κληρονομείται με τον επικρατούντα αυτοσωματικό τύπο.

Οι πολύποδες δεν υπάρχουν κατά τη γέννηση, αλλά αρχίζουν να παρουσιάζονται περίπου από το δέκατο τρίτο έτος με επιταχυνόμενο ρυθμό, έτσι ώστε κατά το εικοστό πρώτο έτος ολόκληρο το παχύ έντερο καλύπτεται από εκατοντάδες ή και χιλιάδες πολύποδες. Άτομα με οικογενή πολυποδίαση, αν δεν αντιμετωπισθούν έγκαιρα, ή αν δεν πεθάνουν νωρίτερα από άλλη αιτία, θα αναπτύξουν καρκίνο του παχέος εντέρου 100% και η μέση ηλικία θανάτου των ασθενών αυτών είναι το τεσσαρακοστό πρώτο έτος.

Στην παρούσα εργασία το υλικό που χρησιμοποιήθηκε ήταν 6 παρασκευάσματα ολικής κολεκτομής. Οι 4 ασθενείς ήταν άνδρες και οι 2 γυναίκες. Το ηλικιακό φάσμα ήταν από 19-45 έτη. Σε 2 από αυτά βρέθηκε διηθητικό αδενοκαρκίνωμα παχέος εντέρου χωρίς μεταστάσεις στους επιχώριους λεμφαδένες. Ο ένας ασθενής είχε μάλιστα διεστιακό καρκίνωμα, ενώ μία ασθενής είχε αδενώματα με σοβαρού βαθμού δυσπλασίες.

A (Παθολογική Ανατομική) - 3

Οισοφάγος Barrett

Δήμητρα Κορακάκη

Γ.Ν.Θ. Παπαγεωργίου

Ο οισοφάγος Barrett είναι η μετάπλαση του επιθηλίου του βλεννογόνου του οισοφάγου από πολύστιβο πλακώδες σε κυλινδρικό μονόστιβο με παρουσία καλυκοειδών κυττάρων. Πρόκειται για μια προκαρκινική αλλοίωση, η οποία αυξάνει την πιθανότητα εμφάνισης αδενοκαρκινώματος του οισοφάγου κατά 30 έως 40 φορές.

Παρατηρείται κυρίως σε περιπτώσεις χρόνιας έκθεσης του οξέος του στομάχου λόγω της παλινδρόμησής του από το στομάχι προς τον οισοφάγο (γαστροοισοφαγική παλινδρομική νόσος).

Σκοπός: της εργασίας αυτής είναι η μελέτη των περιστατικών οισοφάγου Barrett στο

Παθολογοανατομικό εργαστήριο του νοσοκομείου Παπαγεωργίου, σε σχέση με την ηλικία, το φύλο, τον τύπο και την εξέλιξή του σε αδενοκαρκίνωμα την τελευταία διετία.

Υλικό και μέθοδος: Κατά την χρονική περίοδο περίπου δύο ετών (από 7/9/2010, έως 9/4/2012), παρουσιάστηκαν στο τμήμα 11 περιστατικά οισοφάγου Barrett. Η ταξινόμηση έγινε κατά ηλικία και φύλο και με βάση τον τύπο μετάπτωσης του πλακώδους επιθηλίου.

Αποτελέσματα: Από τα 11 περιστατικά οισοφαγίτιδας Barrett οι άνδρες ήταν 9 και οι γυναίκες 2. Έξι από αυτούς που εμφάνισαν τη νόσο ήταν ηλικίας από 30 έως 60 ετών, τρεις άνω των 60 και 2 από 17 έως 30. Η μεταπλασία του βλεννογόνου σε 4 περιπτώσεις ήταν τύπου καρδίας, σε 3 τύπου θόλου, σε 2 τύπου σώματος και η μία τύπου άντρου.

Συμπεράσματα: Από την παρούσα μελέτη παρατηρούμε ότι η πλειονότητα των περιστατικών με οισοφαγίτιδα Barrett αφορά άνδρες, μέσης ηλικίας, ενώ η μεταπλασία του βλεννογόνου ποικίλλει.

A (Παθολογική Ανατομική) - 4

Το παθολογοανατομικό τμήμα του νοσοκομείου Παπαγεωργίου: Ένα σύγχρονο εργαστήριο.

Στυλιανός Κατσαρός, Χριστίνα Ανδρίτσου, Χαράλαμπος Πουρνάρης, Δήμητρα Κορακάκη, Θεόδωρος Βλαχόπουλος

Γ.Ν.Θ. Παπαγεωργίου

Το συγκεκριμένο τμήμα του νοσοκομείου Παπαγεωργίου με βάση την αρχιτεκτονική δομή και τη λειτουργία του κτηριολογικού προγράμματός του αποτελεί ένα πρότυπο εργαστήριο, αποδίδοντας μια ταυτότητα η οποία συνδέεται με το περιβάλλον και την τεχνολογία, παρέχοντας παράλληλα άνεση και βελτιωμένες συνθήκες εργασίας στους χρήστες του.

Το κτήριο είναι χωροθετημένο σε ιδεατό οικόπεδο ώστε να μπορεί να λειτουργήσει και ως αυτόνομο, αποτελώντας ανεξάρτητη μονάδα και ως πτέρυγα του νοσοκομείου. Βρίσκεται δίπλα στα χειρουργεία, ώστε να υπάρχει η δυνατότητα άμεσης παραλαβής των παρασκευασμάτων που έρχονται από αυτά, και να γίνεται η πραγματοποίηση ταχείων βιοψιών.

Οι εσωτερικοί χώροι είναι μελετημένοι και προσαρμοσμένοι έτσι ώστε να εξυπηρετούν τις ανάγκες του εργαστηρίου ανάλογα με τις εργασίες που διενεργούνται σε κάθε χώρο, με σκοπό την ταχύτητα διεκπεραίωσης αυτών και την αποφυγή σφαλμάτων και ατυχημάτων από τους εργαζομένους.

Η υγιεινή και η ασφάλεια των εργαζομένων είναι κυρίαρχο ζήτημα για την ποιότητα ζωής σε κάθε εργασιακό χώρο. Στο νοσοκομείο μας λαμβάνονται όλοι εκείνοι οι κανόνες ασφαλείας που προβλέπει ο νόμος, ώστε να προληφθούν και να μειωθούν δραστικά οι κίνδυνοι ατυχημάτων.

Η παροχή του απαραίτητου και σύγχρονου εξοπλισμού από τον φορέα, η σωστή χρήση και συντήρησή του, η πυρασφάλεια, η ύπαρξη δεξαμενών λήψης και αποθήκευσης των τοξικών και μολυσματικών αποβλήτων και η κατάλληλη σήμανση, εξασφαλίζουν ένα ασφαλές και υγιές περιβάλλον εργασίας.

Το τμήμα απαρτίζεται από ένα έμπειρο και εξειδικευμένο προσωπικό του οποίου η κατάρτιση και εκπαίδευση είναι συνεχής. Συμμετέχει ενεργά σε εκδηλώσεις, ημερίδες, σεμινάρια επιμόρφωσης, ερευνητικές προσπάθειες, καθώς και σε ομιλίες σε συνέδρια.

A (Αιματολογία)

IN VITRO Διάλυση των σωρών αιμοπεταλίων με τη χρήση πρωτεολυτικών ενζύμων (πρόδρομη ανακοίνωση)

Βασιλική Κωσταντινίδου, Αλέξιος Τσοκόπουλος, Φαίδρα Ελευθερίου, Αναστασία Σερεμετίδου, Ελένη Βαγδατλή

Αιματολογικό Εργαστήριο, Τμήμα Ιατρικών Εργαστηρίων, Α.Τ.Ε.Ι. Θεσσαλονίκης

Εισαγωγή: Ένα από τα μεγαλύτερα προβλήματα στη «γενική εξέταση αίματος» στους αυτόματους αιματολογικούς αναλυτές, είναι το σφάλμα στην καταμέτρηση των αιμοπεταλίων, όταν αυτά για διάφορους λόγους σχηματίζουν σωρούς.

Σκοπός της παρούσας μελέτης είναι η ανεύρεση μιας ουσίας η οποία θα διαλύσει τους σχηματισθέντες σωρούς αιμοπεταλίων ώστε να καταστεί δυνατή η ακριβής καταμέτρησή τους.

Υλικό - μέθοδος: Σε οκτώ δείγματα αίματος τα οποία εμφάνιζαν ψευδοθρομβοπενία λόγω της ύπαρξης σωρών αιμοπεταλίων έγινε προσπάθεια διάλυσης των σωρών με τη χρήση των πρωτεολυτικών ενζύμων: 1) στρεπτοκινάση (Στρεπτοκινάση από β αιμολυτικό στρεπτόκοκο Lancefield 10 KU), 2) πλασμίνη 150 UG και 3) πρωτεϊνάση (Proteinase bacterial 50 mg). Για την επίτευξη του βέλτιστου pH δράσης των ενζύμων, καθένα από αυτά αραιώθηκε με ρυθμιστικό διάλυμα οξικού οξέος και οξικού νατρίου (σύμφωνα με τις οδηγίες του κατασκευαστή τους). Για καθένα από τα παραπάνω ένζυμα έγιναν πέντε αραιώσεις (α, β, γ, δ, ε) με προοδευτικά μειούμενες ποσότητες ενζύμου αρχής γενομένης με 1) 50μl στρεπτοκινάσης, 2) 3μl πλασμίνης και 3) 5μl πρωτεϊνάσης. Κάθε αραιώση ενζύμου προστέθηκε σε 200μl αίματος. Τα δείγματα αίματος με τις αντίστοιχες αραιώσεις των τριών ενζύμων μετρήθηκαν στον αιματολογικό αναλυτή άμεσα και μετά από 30, 60 και 120min.

Αποτελέσματα: Στη «γενική αίματος» των δειγμάτων στα οποία προστέθηκε στρεπτοκινάση και πλασμίνη δεν υπήρξε αύξηση του αριθμού των αιμοπεταλίων σε καμία αραιώση ενζύμου και με την παρατήρηση επιχρισμάτων επιβεβαιώθηκε η παραμονή των αιμοπεταλιακών σωρών. Σε τέσσερα από τα 8 άτομα η πρωτεϊνάση προκάλεσε διάλυση του 50% περίπου των σωρών μετά από 120min στις μικρότερες ποσότητες ενζύμου (γ₃:3μl, δ₃:2μl, ε₃:1μl).

Συμπεράσματα: Η στρεπτοκινάση και η πλασμίνη, αν και έχουν in vivo ινωδολυτική δράση, δεν μπορούν να προκαλέσουν διάλυση των in vitro σωρών αιμοπεταλίων. Αντίθετα, η προσθήκη μικρής ποσότητας πρωτεϊνάσης προκαλεί μερική διάλυση των σωρών. Απαιτείται περαιτέρω έρευνα με νέες αραιώσεις και επανέλεγχο των δειγμάτων μετά από περισσότερη ώρα.



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