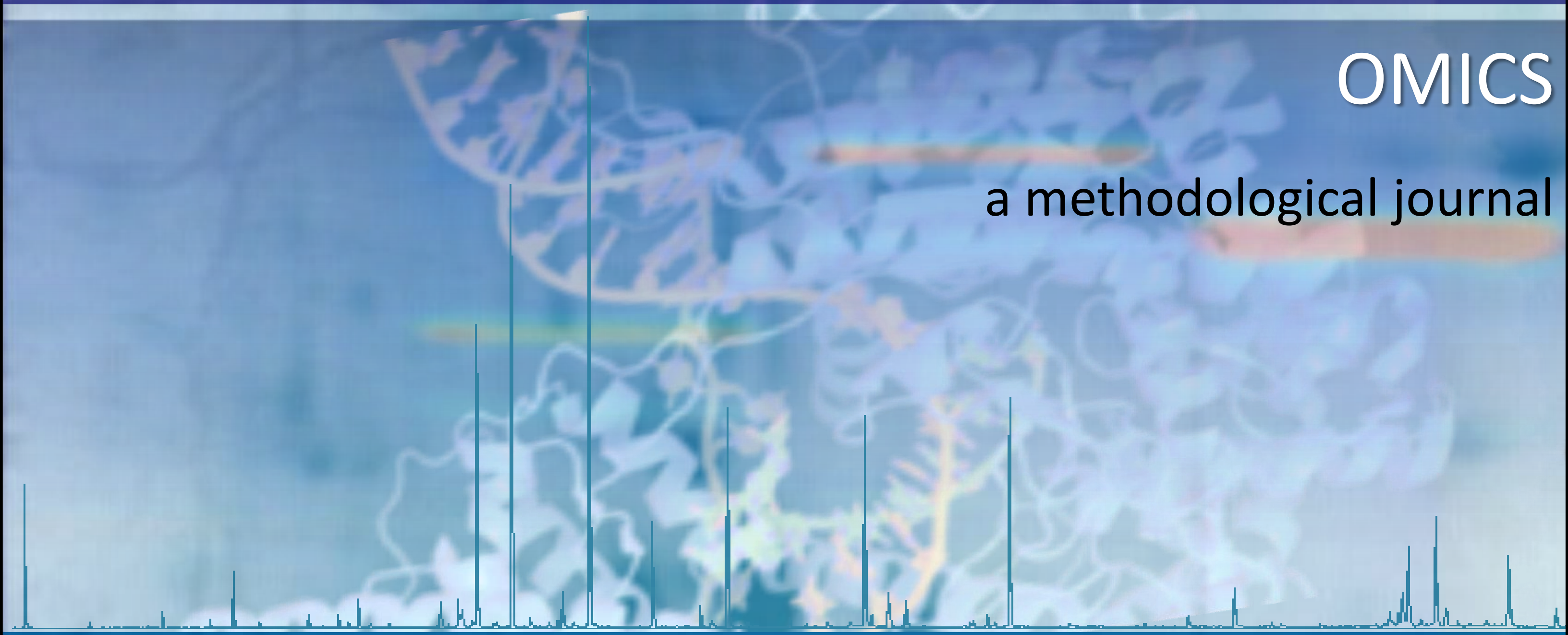
Volume 7 | Issue 2 | December 2017 ISSN 2182-0287

Journal of Integrated OMICS

a methodological journal



JIOMICS

Journal of Integrated OMICS

Focus and Scope

Journal of Integrated OMICS, JIOMICS, provides a forum for the publication of original research papers, preliminary communications, technical notes and critical reviews in all branches of pure and applied "-omics", such as genomics, proteomics, lipidomics, metabolomics or metallomics. The manuscripts must address methodological development. Contributions are evaluated based on established guidelines, including the fundamental nature of the study, scientific novelty, and substantial improvement or advantage over existing technology or method. Original research papers on fundamental studies, and novel sensor and instrumentation development, are especially encouraged. It is expected that improvements will also be demonstrated within the context of (or with regard to) a specific biological question; ability to promote the analysis of molecular mechanisms is of particular interest. Novel or improved applications in areas such as clinical, medicinal and biological chemistry, environmental analysis, pharmacology and materials science and engineering are welcome.

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JOURNAL OF INTEGRATED OMICS

A methodological Journal

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JOURNAL OF INTEGRATED OMICS
A METHODOLOGICAL JOURNAL
HTTP://WWW.JIOMICS.COM

ORIGINAL ARTICLE | DOI: 10.5584/jiomics.v7i2.218

Linguistic Difficulties for Translators and Interpreters: The Case of Forensic Law and Science Documents

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Received: 17 November 2017 **Accepted:** 19 December 2017 **Available Online**: 19 December 2017

ABSTRACT

Forensic Science (or Forensics) holds various fields of science (Psychology, Pathology, Odontology, Toxicology, Digital Forensics, to cite a few) involved in solving crimes and offences at any stage in criminal proceedings and researches. On the other hand, Forensic Law is a legal branch that involves issues related to forensic techniques in a justice system (being thus linked to Criminology, Criminal Law, and Civil Law). The intersection of Science and Law contributes to finding out the truth of a case, either criminal or civil, and they both have undergone dramatic progress in recent years. The aforementioned intersection provides specialists with a considerable amount of documents, either electronic or paperback…and they often need to be translated or interpreted into other languages. The high level of technical terms, complex nouns and phraseology, hamper their translation, interpreting, and proofreading services. This paper, will provide the reader with the main linguistic features of Forensic Science and Law Discourse, matched with source texts and target texts. By means of the use of original Forensic documents, translators and interpreters will be provided with techniques and strategies for facing their translation and proofreading.

Keywords: Forensic Science; Forensic Law; Linguistic difficulties; Translation; Proofreading .

1. Introduction

As recently stated and highlighted throughout the 1st International Conference on Translational Forensics (Caparica, 2017), forensic sciences and technology (Forensics) are of utmost importance for court cases (civil and criminal law). Owing to this, the amount of research and casework documents is becoming increasingly demanded among researchers, laboratory workers, and legal professionals. Undoubtedly, Forensics is at the crossroads for Science, Technology and Law.

By considering human migration, and international cooperation, the noteworthy amount of relevant available sources –both scientific and legal– requires the intervention of highly skilled translators, interpreters and proofreaders. This is partly because many of the key documents do present both scientific and legal linguistic features. Consequently, the professional translator may deal with “hybrid documents” of a mixed nature. These text genres entail two-fold difficulties: on the one hand, they describe scientific procedures and techniques, on the other they must show the reader that they comply with all rules and legislations in force.

In this paper, several documents related to forensic science and law discourse, will be selected for identifying their linguistic difficulties and the applicable translation techniques. Our final goal is to generate a corpus selective list, see whether they entail translation difficulties and provide the target reader with all the solutions available.

1.1. Specialized Translation: Techniques

Specialized translation (science, technology, law, business) differs from general translation (newspaper, advertisement) by providing linguistic solutions to highly specialized texts and documents. As it has been previously researched [1, 2, 3, 4], the task of specialized translators is hence challenging, and problems will emerge during the translation process, since the list of required skills is noteworthy:

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- linguistic skills
- translation skills
- thematic area knowledge
- parallel documentation knowledge
- computer sciences for translators and proof-readers.

Due to the difficulties encountered by translation professionals, many scholars suggested translation techniques aimed at providing them with linguistic instruments for their professional task. Below is the overall list coined by Hurtado Albir [5], and Molina and Hurtado Albir [6].

Classification of translation techniques:

Adaptation: To replace a source text cultural element with one from the target culture. Baseball (E) ⇒ Fútbol (Sp).

Amplification: To introduce details that are not formulated in the ST: information, explicative paraphrasing, (A) Ramadan ⇒ Ramadan, the Muslim month of fasting (E).

Borrowing: To take a word or expression straight from another language. Pure borrowing: Lobby (E) ⇒ Lobby (Sp), Naturalized borrowing: Meeting (E) ⇒ Mitin (Sp).

Calque: Literal translation of a foreign word or phrase; it can be lexical or structural. École normale (F) ⇒ Normal School (E).

Compensation: To introduce an element of information or stylistic effect in another place in the target text because it cannot be reflected in the same place as in the original text. I was seeking thee, Flathead (E) ⇒ En vérité, c'est bien toi que je cherche, O Tête-Plate (F).

Description: To replace a term or expression with a description of its form or/and function. Panettone (I) ⇒ The traditional Italian cake eaten on New Year's Eve (E).

Discursive creation: To establish a temporary equivalence that is totally unpredictable out of context. Rumble fish (E) ⇒ La ley de la calle (Sp).

Established equivalent: To use a term or expression recognized (by dictionaries or language in use) as an equivalent. They are as like as two peas (E) ⇒ Se parecen como dos gotas de agua (Sp).

Generalization: To use a more general or neutral term. Guichet, fenêtre, devanture (F) for window (E).

Linguistic amplification: To add linguistic elements. This is often used in consecutive interpreting and dubbing. No way (E) ⇒ De ninguna de las maneras (Sp).

Linguistic compression: To synthesize linguistic elements in the TT. This is frequently used in simultaneous interpreting and in sub-titling. Yes, so what? (E) ⇒ ¿Y? (Sp).

Literal translation: To translate a word or an expression word for word. She is reading (E) ⇒ Ella está leyendo (Sp).

Modulation: To change the point of view, focus or cognitive category in relation to the source text. Estás embarazada (Sp) ⇒ You are going to have a child (E).

Particularization: To use a more precise or concrete term. Window (E) ⇒ Guichet, fenêtre, devanture (F).

Reduction: To suppress a ST information item in the TT. Ramadan, the Muslim month of fasting (Sp) ⇒ Ramadan (A).

Substitution (linguistic, paralinguistic): To change linguistic elements for paralinguistic elements (intonation, gestures) or vice versa. To put one's hand on the heart (A) ⇒ Thank you (E).

Transposition: To change a grammatical category. He will soon be back (E) ⇒ No tardará en venir (Sp).

Variation: Introduction or change of dialectal indicators, changes of tones.

2. Material and Method

The corpus analyzed consisted of hybrid documents linked to Forensics that entails translation difficulties due to its hybrid (scientific and legal) nature. The documents researched (or text genres) amounted to 15, as follows below:

- 5 Death Certificates.
- 5 Advanced Medical Directives (also known as Living Wills).
- 5 Informed Consents (to Surgery/Special Procedure).

Death Certificates (or Certificates of Death) are documents stating the particulars on an individual who has just deceased. The structure follows a table format, stating data such as the name (first, middle and last) of the deceased (also called "decedent"), the date and place of death, her/his sex and age, her/his occupation, and the cause of death. A doctor certifies the cause of death, although other relevant figures appear in this forensic document:

- the informant (in charge of reporting the death),
- the coroner (responsible for confirming and certifying the death within the premises of a jurisdiction),
- the registrar (or officer who certifies that the document issued is a true copy of the original entry).

As far as the Advanced Medical Directive is concerned, it states the decisions of an individual regarding his consent (or refusal) to medical treatment. The document presents alternative naming (Living Will, Advance Directive, Advance Decision, Advance Decision to Refuse Treatment (ADRT) o Advance Health Care Directive). The format follows a four-fold structure, by combining the following sub-documents:

Advanced Medical Directive execution
Medical Treatment
Power of Attorney for Health Care
Organ and Tissue Donation.

The relevant figures interacting throughout the document are also diverse:

- the person executing the document (the granter)
- the healthcare providers and the doctors (who shall be in agreement on the diagnosis of a terminal condition of the granter)
- the healthcare agent ("the agent") who shall make health care decisions based on the details given in the Directive.

The Informed Consent (to Surgery/Special Procedure) is a multi-page document that provides the patient with the information needed so that s/he may give or refuse her/his consent to the surgery or special procedure recommended by the doctors. The involved parties are three: the patient (giving or refusing consent to the recommended surgery/ special procedure) or her/his legal representative; the doctors assisting the patient (and recommending the operation or procedure); the practitioner (who is performing the procedure or administering the medical treatment). There could also appear an interpreter providing language assistance for foreign citizens.

The document sections may include the following:

a) The consent to the surgery or procedure described therein, along with its benefits, risks and adverse reactions
b) The physician certification
c) The consent to blood transfusion
d) The interpreter's statement, if applicable.

Our research methods made full use of the translation techniques coined by Hurtado (and Molina Hurtado).

The texts listed above were thoroughly analyzed in search of translation difficulties. Then, the translation difficulties were selected and solved by using the translation techniques described hereby. Subsequently, the results of the study were structured presenting the difficulties' solutions by means of illustrative examples selected from the corpus.

3. Results

After the analysis of the corpus, multiple linguistic elements that could represent a "troublesome item" for translators and interpreters were identified. The list was shaped as stated below:

- Adaptation: "First Name, Middle Name and Last Name" [in the three text genres] for "Primer nombre de pila, Segundo nombre de pila y Apellido".
- Amplification [text: Advanced Medical Directive]: "Pain" for "Malestar y dolor", "Being of sound mind" for "Estando en pleno uso de mis facultades mentales"; [text: Informed Consent]: "the undersigned" for "el abajo firmante" or "el signatario del documento"; [text: Death Certificate and Informed Consent]: "Physician's Certificate" for "Informe Pericial del Facultativo".
- Borrowing: "Test" [in the three text genres] for "Test"; "Doctor" for "Doctor".
- Calque and Literal translation [text: Informed consent]: "the operation or procedure" for "la operación/ intervención o procedimiento", "adverse reactions" for "reacciones adversas".
- Compensation: "Date and Place" [in the three text genres] should be replaced by "Lugar y Fecha"; [text: Advanced Medical Directive]: "for the purpose of transplant only" for "con el fin único del transplante de los mismos".
- Description: [in the three text genres]: "print name" for "en letras mayúsculas/con letra de imprenta/con letra de molde".
- Established equivalent [text: Death Certificate]: "Qualification" for "En calidad de", "Registration Number" for "Número de Colegiado", "Marital status" for "Estado civil", "Occupation" for "Profesión", "Informant" for "Declarante", "true copy" for "copia fiel", "in a register in my custody" for "que obra en mi poder"; [text: Advanced Medical Directive and Informed Consent]: "Sections" for "Cláusulas"; [text: Informed Consent]: "member" for "extremidad"; [text: Advanced Medical Directive]: "Advanced Medical Directive": "Declaración de Voluntades Anticipadas" (which is also a form or Particularization), "firm and settled decisions" for "la expression formal de mi voluntad", "in my bests interests" for "en mi (propio) bien".
- Generalization [text: Death Certificate]: "Certified copy of an entry" for "Certificado de Defunción".
- Modulation [text: Death Certificate]: "Deceased/ Decedent" for "Causante".
- Particularization [text: Informed Consent]: "terms and conditions" for "cláusulas", "foregoing" for "el presente (documento)"; [text: Death Certificate]: "the particulars above" for "la información consignada anteriormente".
- Reduction [text: Informed Consent]: "Warranty or guarantee" for "Garantía", "but are not limited to" for "entre otras"; [text: Advanced Medical Directive]: "Cannot be cured" for "Irreversible", "Under undue influence, duress or menace" for "sin coacción (alguna)", "Where I am unable to communicate my wishes" for "Que no pueda expresar mi voluntad"; text: Death Certificate]: "Maiden surname of woman who has married" for "Apellido de soltera", "the employment followed by the deceased" for "el empleo del fallecido/ causante".
- Transposition [text: Informed Consent]: "The hospital maintains" for "El mantenimiento del hospital".

4. Discussion

Within the forensic corpus analyzed, a considerable amount of binomial and trinomial expressions has been perceived, usually placed within legal documents. Examples of these are the following: "You authorize and consent" (text: Informed consent), "accurately and completely" (Informed Consent), "being of sound and disposing mind, memory and understanding" (Advanced Medical Directive), "operation or procedure" (Informed Consent), "terms and conditions" (Informed Consent), "disease or condition" (Death Certificate), "to the best of my knowledge and belief" (Death Certificate), "firm and settled decisions" (Advanced Medical Directive), to cite a few.

Obviously, interpreting techniques, such as Substitution, Linguistic Amplification, and Linguistic Compression were not detected within the corpus. Additionally, Variation would be necessary when the translator receives a translation brief requiring her/him to perform a translation for a different Spanish-speaking country. Discursive creation, a common technique for literary and audiovisual translation, was not required for our goals. Nevertheless, "dynamic translation" was required when a lack of exact equivalences was identified. As an example, "Registrar" is not "Registrador" (used for "Registrador de la Propiedad"), is a type of civil or public servant in charge of a Civil Registry, therefore, they are talking about a "Responsible/Jefe del Registro" in Spain; "the Physician" is not "el Físico", but "El Médico/Especialista".

Euphemisms were also tagged in the researched corpus:

- "Condition" with the meaning of "Disease/Illness": Enfermedad, Patología
- "To die" would become "fallecer" in Spanish.

Meanwhile, the term "Death" (from a "Death Certificate") has multiple translations into Spanish:

- "(Certificado/Certificación de) Defunción" for the document's name
- "(Causa del) Óbito" for the cause ("death caused by")
- "Acta" or "Partida" for the document's Register number.

The "Informed Consent" makes full use of variety when dealing with the biomedical specialist, herein called "physician", "doctor", "practitioner" and "medical practitioner". Nonetheless, there are linguistic alternatives in Spanish language: "especialista", "medico", and "facultativo".

In order to summarize the translation techniques identified within our corpus, several tables (Table 1, Table 2, Table 3, and Table 4 for overall results) were designed to illustrate the results of our research, as stated below. A cross was inserted when a translation technique proved useful within each text.

Firstly, Table 1 will show the translation techniques identified within the corpus of Death Certificates.

Table 1. Corpus Translation Techniques: Death Certificates .

Translation Techniques	Death Certificates
Adaptation	X
Amplification	X
Borrowing	X
Calque and Literal Translation	
Compensation	X
Description	X
Discursive Creation	
Established Equivalent	X
Generalization	X
Linguistic Amplification	
Linguistic Compression	
Modulation	X
Particularization	X
Reduction	X
Substitution	
Transposition	
Variation	

Secondly, Table 2 will summarize the translation techniques identified within the corpus of Advanced Medical Directives.

Table 2. Corpus Translation Techniques: Advanced Medical Directives .

Translation Techniques	Advanced Medical Directives
Adaptation	X
Amplification	X
Borrowing	X
Calque and Literal Translation	
Compensation	X
Description	X
Discursive Creation	
Established Equivalent	X
Generalization	
Linguistic Amplification	
Linguistic Compression	
Modulation	
Particularization	
Reduction	X
Substitution	
Transposition	
Variation	

Thirdly, Table 3 will illustrate the translation techniques identified within the corpus of Informed Consents.

Table 3. Corpus Translation Techniques: Informed Consents.

Translation Techniques	Informed Consents
Adaptation	X
Amplification	X
Borrowing	X
Calque and Literal Translation	X
Compensation	X
Description	X
Discursive Creation	
Established Equivalent	X
Generalization	
Linguistic Amplification	
Linguistic Compression	
Modulation	
Particularization	X
Reduction	X
Substitution	
Transposition	X
Variation	

Table 4. Corpus Translation Techniques: Overall Results.

Translation Techniques	Death Certificates	Advanced Medical Directives	Informed Consents
Adaptation	X	X	X
Amplification	X	X	X
Borrowing	X	X	X
Calque and Literal Translation			X
Compensation	X	X	X
Description	X	X	X
Discursive Creation			
Established Equivalent	X	X	X
Generalization	X		
Linguistic Amplification			
Linguistic Compression			
Modulation	X		
Particularization	X		X
Reduction	X	X	X
Substitution			
Transposition			X
Variation			

Lastly, Table 4 will show the comparison of the translation techniques identified within Death Certificates, Advanced Medical Directives, and Informed Consents.

5. Concluding Remarks

Both Death Certificates and Informed Consent allowed us to use 10 translation techniques, whereas Advanced Medical Directives needed 7 techniques. The Informed Consent was the document with an increased usage of binomials and trinomials. Since it entails a large amount of legal phraseology, this was a foreseeable feature. Nevertheless, Death Certificates and Advanced Medical Directives also made full use of these grammatical expressions. Hence, either binomials plus trinomials could be balanced by looking for a cultural (established) equivalent, or they could undergo reduction ("last will and testament" for "testament"). Translation assessment is a troublesome task, although there are several techniques that could be used when facing the translation task. Euphemisms could be translated into other euphemisms (established equivalent: "deceased" for "causante") or be transferred by means of Calques and Literal Translations ("difunto"). Borrowings ("testar" from "to test") do not always work, since "the doctor" sometimes means "el doctor", but other times it refers to "the physician". Compensation is advisable for non-literal translations, but not for "calqued translations". Adaptation is affordable when the target culture holds a cultural equivalent. Amplification is a useful technique for translating simple technolects into complex terminology. It is recommended that translators draw on Descriptions for items ("coroner") with the lack of lexical equivalent in the target culture. Indeed, Established Equivalents fit when each culture utters differently the surrounding reality. Generalization may be used when translators find a more general (target) term. Translating cultures often requires the use of Modulation. Particularization is always a kind of "itemation" with the aim of providing the target reader with more specific terms. The translator often compensates the number of words by resorting to words' Reduction. Finally, Transposition is compulsory when the grammatical category of a term requires replacement for an alternative category. Overall, when a professional translator opts for a technical term, s/he should be coherent to her/his translation, especially when dealing with the translation of document´s headings.

Translators and interpreters should also work hand in hand with other (scientific and legal) experts. When translating a court text, the judge or magistrate would provide relevant data. If the translator faces the translation of a forensic text, the forensic scientist or technician would supply her/him with the information required.

Undoubtedly, the use of parallel documentation is a 'must' for the translator's desk (they often provide us with established or dynamic equivalents) and the document's dossier should always be enlarged and updated. Bearing in mind that forensic documentation is complex, groundbreaking, and highly relevant, translators and interpreters must make full use of the wide variety of techniques researched herein. Skilled translators and interpreters, who shall persistently actualize their knowledge, must face the discursive hybrid intersection of Forensic Science and Law, always challenging and enthralling, that requires likewise hybrid translation skills and abilities.

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SPECIAL ISSUE

SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

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JIOMICS | VOL 7 | ISSUE 2 | DECEMBER 2017 | 7 - 40

JOURNAL OF INTEGRATED OMICS
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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Tolerance to caspofungin in *Candida albicans* is associated with at least three distinctive mechanisms that govern expression of *FKS* genes and cell wall remodeling

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ABSTRACT

Fungal infections are currently found to be a leading cause of global infectious disease, resulting in death tolls surpassing those from such diseases as drug-resistant tuberculosis or malaria. Infections involving *Candida* species are responsible for most systemic invasive fungal infections (1). Despite an increase in the diversity of *Candida* species isolated from clinical samples, *C. albicans* is still the predominant cause of infections causing up to 50% of candidemia (2, 3). *C. albicans* also prevails among isolates from blood stream, from 42% to 50.7% (4). Currently, echinocandins (ECNs) that prevent biosynthesis of cell wall are recommended for treatment of *Candida* infections, as front-line drugs. Expanding echinocandin use to prevent or treat invasive fungal infections has led to an increase in the number of breakthrough infections due to resistant Candida species. ECN resistance is well documented for *C. albicans*. The genome of *C. albicans* contains three homologous *FKS* (FK506 sensitivity) genes: *FKS1* (*GSC1*) (orf19.2929), *FKS2* (*GSL2*) (orf19.3269), and *FKS3* (*GSL1*) (orf19.2495). The only generally accepted mechanism of *C. albicans* clinical resistance to ECNs involves point mutations in FKS1 (5). Such clinically resistant strains usually exhibit high minimum inhibitory concentration (MIC) values for ECNs. However, the mutations in *FKS1* gene are not sufficient to explain elevated ECN MICs that are observed in many clinically-derived *C. albicans* strains with no *FKS1* mutations (6). A better understanding is needed to assess the cellular factors that promote decrease of susceptibility to ECNs and ultimate breakthrough.

We find that, following direct selection on caspofungin (CAS) from the ECN class of drugs, laboratory resistance (tolerance) can be conferred by at least three independent mechanisms: 1) reversible loss of one chromosome 5 (Ch5); 2) conversion of one Ch5 into a chromosome with two right arms, and 3) an aneuploidy-independent mechanism that downregulats 2-fold or more ~9.6% of genes on disomic Ch5s that are also downregulated on the monosomic Ch5 (6; E. Rustchenko, unpublished RNA-seq data). Mutants selected for CAS tolerance possessed remodeled cell walls with elevated chitin and showed overall downregulation of *FKS* genes that were free of mutations (7). We also found that Ch5 carries at least three genes *CSU51* (orf19.1105.2), *CHT2* (orf19.3895), and *PGA4* (orf19.4035) for negative regulation of ECN susceptibility. These genes were downregulated in all kinds of mutants irrespectively of Ch5 ploidy. The final number of genes dor negative control of ECN susceptibility is yet to be established (8). In addition, Ch5 carries at least two genes *CNB1* and *MID1* involved in calcineurin signaling pathway that encode positive regulators of ECN susceptibility. These genes are expressed at the disomic level in all kinds of mutants irrespectively of Ch5 ploidy. The final number of Ch5 genes for positive control of ECN susceptibility is also not known yet. Apparently, multiple mechanisms can impact the relative expression of genes residing on Ch5 or outside Ch5 controlling the genes in a similar manner. Although mutations in *FKS1* have previously been associated with CAS resistance, we find mechanisms of CAS tolerance that are independent of mutations in *FKS1* suggesting it is an earlier event in resistance development.

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Acknowledgments: This work was supported in part by National Institutes of Health grant AI110764 to E.R. and grant AI109025 to D.S.P; Merck Sharp & Dohme Inc. MISP award 51184; and The University of Rochester Funds to E. Rustchenko, and National Natural Science Foundation of China (no. 81173100, 81273556) to Yongbing Cao.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Profiling the dynamics of the metabolism of human cells: A unique approach to illuminating human disorders

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ABSTRACT

We employed plates PM M1-M8 of the Phenotype MicroArrays (PM) platform from Biolog (Haywood, CA) to explore cell utilization of single compounds as an energy source or metabolic effectors in patients with various human genetic disorders with known gene mutations. Doing so, allowed us to generate kinetic profiles which created distinct fingerprints for some human disorders.

We have successfully applied the PM methodology to establish: 1) Abnormal utilization of critical cytokines mediating the immune response of both fibroblasts and lymphoblasts from patients with a duplication of *MECP2* and recurrent infections; 2) Low utilization of Krebs cycle compounds and high utilization of sugars in patients with Snyder-Robinson syndrome; 3) Patients with the FG syndrome and with Lujan syndrome exhibited different metabolic profiles even though both have mutations in the same gene, *MED12*; 4) Abnormal response to growth factors of fibroblasts from affected tissue from patients with segmental overgrowth. Based on these observations we have explored the utilization of specific compounds to treat somatic overgrowth conditions and have obtained some success in vitro.

The PM assay represents a novel approach to investigate the cellular metabolism and evaluate the functional impact of genetic alterations on metabolic pathways. The assay does not require any prior knowledge of how a particular gene may affect a metabolic pathway. Most importantly, the findings may point to an avenue of potential treatment otherwise not evident from the information known on the function of the gene.

Keywords: Metabolic profiling; human genetic syndromes; Phenotype Microarray

Acknowledgments: Grants from NICHD (R21HD072473), NINDS (R01NS073854) and South Carolina Dept. of Disabilities and Special Needs (SCDDSN)

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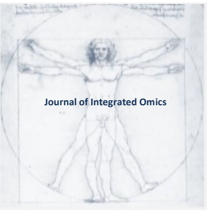

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Combining activity-based and substrate-based profiling approaches in the characterization of a/b-hydrolase domain (ABHD) containing serine hydrolases

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Available Online: 2 October 2017

Abstract

The human metabolic serine hydrolases (mSHs) comprises a large family of enzymes and utilize a conserved serine nucleophile to hydrolyze e.g. amide, ester and thioester bonds. The majority of mSHs contains an α/β-hydrolase domain (ABHD) fold and use a Ser-His-Asp (SHD) triad for catalysis. Genes encoding ABHD proteins are found throughout the reported genomes and conserved structural motifs shared by these proteins are predictive for common roles in cell signaling and lipid metabolism. Although a handful of these enzymes are well-known, many still remain poorly characterized with respect to their physiological substrates, products and metabolic functions. Recent research have brought mSHs such as monoacylglycerol lipase (MAGL), acyl protein thioesterase 1 (APT1/LYPLA1) and KIAA1363 to the center stage of cancer research. Various aggressive cancer cells show heightened serine hydrolase activity, supporting high migratory, invasive, and protumorigenic activity. Therefore, small molecule inhibitors targeting the mSH family may open new avenues for anticancer therapies. In addition, newly discovered inhibitors could be useful in characterizing biochemical and physiological function of unknown mSHs. Hydrolase activity can be readily monitored using activity-based protein profiling (ABPP), a chemoproteomic approach that relies on the use of catalytic serine-targeting fluorophosphonate probes. The ABPP requires no *a priori* knowledge of the identity of the target and enables parallel activity measurement of many enzymes in complex native proteomes. In competitive ABPP, the proteome is first treated with the inhibitor candidate after which the mSHs are labeled with the fluorescent probe. Protein bands targeted by the inhibitor can be visualized using in gel fluorescence imaging after SDS-PAGE separation. In this presentation, I will highlight the applicability of ABPP when combined together with natural or artificial substrate-based activity profiling in our recent efforts to characterize human ABHD family members, such as ABHD11 [1], ABHD16A/BAT5 [2], ABHD6 and ABHD12 [3] and the cysteine mutants of MAGL [4].

Acknowledgments: Supported by the Academy of Finland, Research grants #139620 and #278212

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Coffee Consumption, Obesity and Reduced Risk of Type-2 Diabetes

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ABSTRACT

Diabesity [1] is the term used to describe the coexistence of type-2 diabetes and obesity, and it is responsible for more than 90% of the world's 382 million people with type-2 diabetes. Sadly, this number is set to rise beyond 592 million in less than 25 years. Innumerous reports from epidemiological studies have been published claiming that regular coffee intake, caffeinated or decaffeinated, averaging 3-4 cups a day, reduces significantly the risk of developing diabetes type 2 [2, 3]. Caffeine is present in the brewed coffee on average at 0.5-2% and has well- known properties, such as thermogenesis and ergogenic properties [4] that could be used to explain part of the effect of coffee in reducing obesity. However, coffee is a very complex mixture and the presence of chlorogenic acids, phenolic antioxidant compounds, is found in much higher concentration than caffeine (7-12%); depending on the beans blend (percentage of basically arabica and robusta species) and the roasting process (the higher temperature and longer roasting process, the lower the amount of those compounds in coffee) [5]

As of today, there are mounting evidences of the reduced risk of developing type-2 diabetes by regular coffee drinkers of 3-4 cups a day. The effects are likely due to the presence of chlorogenic acids and caffeine; the two constituents of coffee in higher concentration after the roasting process [6].

Acknowledgments: The authors would like to acknowledge the fundamental support from Mrs. Valerie Yaughn, Director of the Library at South University, Savannah Campus in helping to find peer-reviewed papers that were of interest. Dr. Darcy Lima passed away last July 2015 and I wanted to acknowledge his infinite encouragement to write this and many other papers, book chapters and books and as research partner and enthusiast. He will be forever remembered.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Fluorescence lifetime based assays in drug discovery

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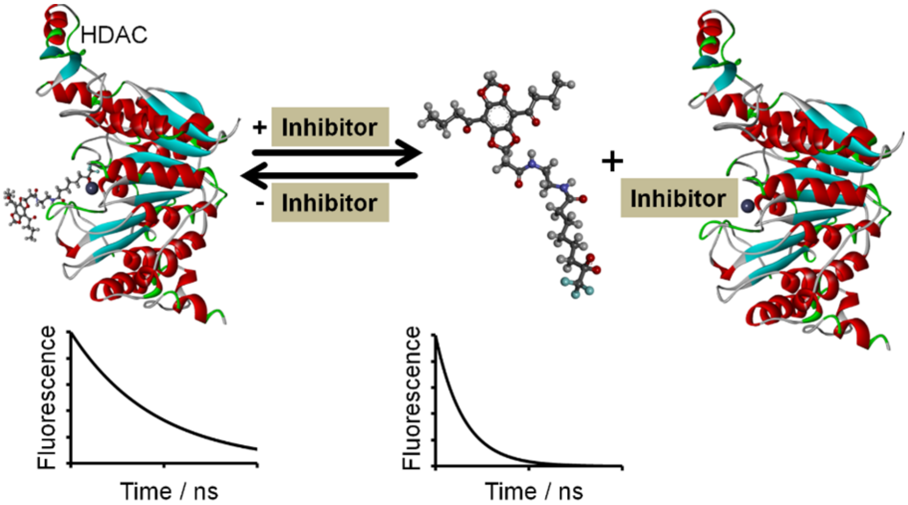
ABSTRACT

High-throughput assays for drug screening applications have to fulfill particular specifications. Besides the capability to identify even compounds with low potency, one of the major issues is to minimize the number of false-positive hits in a screening campaign in order to reduce the logistic effort for the subsequent cherry picking and confirmation procedure. In this respect, fluorescence lifetime (FLT) appears as an ideal readout parameter that is supposed to be robust against autofluorescent and light-absorbing compounds, the most common source of systematic false positives. The extraordinary fluorescence features of the recently discovered [1,3]dioxolo[4,5-f][1,3] benzodioxole dyes were exploited to develop FLT-based binding assays for several bacterial and human isoforms of the histone deacetylase (HDAC) family.[1-2]



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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

MALDI TOF MS Profiling: advances in species identification and future prospects

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Abstract

Matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) based microbial species identification has emerged as an important tool in modern diagnostic laboratories, due to its rapidness, easy handling, cost-effectiveness, high-throughput analysis and reliability as that of genome-based techniques. This technique involves direct transfer of intact microbial colonies on to a target plate, co-crystallised with UV absorbing chemicals referred to as matrix, generation of MALDI spectra and deduction of species through pattern matching with the reference spectra library of well-defined microorganisms. Despite the advancement over past two decades, none of the available reference databases are complete in terms of all known genus/species of microorganisms. Therefore, the commercial software tools include a provision for database extension by creation of additional reference spectra either to improve the identification confidence or to include missing species information. This provision has been reported to be successful in identification of several microbial species, higher organisms such as parasites and their biological vectors, investigation of geographical origin of food products such as mozzarella cheese or differentiation of immune cells. In our laboratory, we extended the commercial database with reference spectra to enable species identification up-to the genotypes of the colourless microalgae genus *Prototheca*, the only known plant like infectious agent associated with rare but severe infections in humans and animals. Subsequently, MALDI based *Prototheca* identification has been included as a rapid tool in our diagnostics, which was otherwise, time-consuming and tedidious [1, 2]. Furthermore, database extension resulted in enhanced identifcation confidence and differentation of the members of the *Staphylococcus intermedius-group* (SIG: *S. intermedius*, *S. pseudintermedius* and *S. delphini*), an important opportunistic pathogens in animals and occasionally in humans [3]. The standard formic acid/acetonitrile producure recommended for microbial protein extraction was successfully applied to compile a reference database for different body parts of the insect Tsetse fly (*Glossina*), biological vector of African sleeping sickness causing trypanosomes [4]. Our work and other recent publications indicate the possibilities of utilising MALDI profiling towards universal identification of microorganisms, insects and other higher organisms. In future, a single MALDI TOF MS measurement might be useful in deriving multiple informations such as species, host or geographical specificity, antimicrobial resistance pattern and presence of pathogens within biological vectors.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Sugar Rush: New LC-MS method to quantify the plant regulator Trehalose-6-Phosphate

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ABSTRACT

Metabolomics is one 'omics' approach that can be used to acquire comprehensive information on the composition of a metabolite pool providing a functional screen of the cellular state. By quantifying the changes taking place inside cells at specific times and under specific conditions, metabolomics offers new insight into cellular biology and a new path of research into the development of abiotic stress tolerant crops [1-2].

Studies of the plant metabolome include the analysis of a wide range of chemical species with diverse physical properties, from ionic inorganic compounds to biochemically derived hydrophilic sugars, organic and amino acids, and a range of hydrophobic lipid-related compounds. Current plant metabolomics studies therefore combine robust on-line chromatographic separations with the high sensitivity and specificity provided by mass spectrometry (e.g., LC-MS, GC-MS) in an effort to acquire more comprehensive metabolite coverage [3].

Trehalose 6-phosphate (Tre6P), the intermediate of trehalose biosynthesis, is an essential signal metabolite in plants. Tre6P is the phosphorylated intermediate of trehalose biosynthesis. It is a signal of Suc status in plants and influences many metabolic and developmental processes, including responses to stress conditions [4]. However, the almost undetectable levels of T6P together with the complex plant matrix and the presence of Tre6P isomers makes the detection of this metabolite challenging. In this presentation, details of our highly sensitive and reliable LC-MS method to detect and quantify Tre6P in the picomole range in Medicago truncatula roots and leaves subjected to water deficit will be discussed [5]. This new analytical tool is fully validated and can now be used to measure low-abundant Tre6P in other biological systems to better understand the regulation of this signaling metabolite.

Acknowledgments: C. António gratefully acknowledges support from the FCT Investigator Programme (IF/00376/2012/CP0165/CT0003) by Fundação para a Ciência e a Tecnologia (FCT) and funding from the ITQB NOVA R&D Unit GREEN-it 'Bioresources for sustainability' (UID/Multi/04551/2013).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Using multi-omics profiling to identify target pathways for the treatment of age-related neurodegenerative diseases

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ABSTRACT

There is currently no drug to prevent of slow the progression of Alzheimer's disease (AD) pathology. Because age is the greatest risk factor for sporadic AD, phenotypic screens based on old age-associated brain toxicities were used to develop new drug candidates based on the natural products curcumin and fisetin (1-4). Since certain aspects of aging may be the primary cause of AD, we hypothesized that these drug candidates would be effective in rapidly aging SAMP8 mice. These mice show a progressive, age-associated decline in brain function similar to human AD patients. To determine if the drug candidates could prevent the progression of age-associated declines in brain function, 8 month old SAMP8 mice were fed the drug candidates in their diet for 4 months. At 12 months of age, changes in behavior, protein expression, the levels of metabolites and the whole transcriptome in mice fed the drug candidates were compared to 8 month old mice and 12 month old mice fed a control diet. Using this inclusive and integrative multi-omics approach, we identified a subset of metabolic changes associated with aging that may be relevant to sporadic AD as well as other forms of dementia. We found that some of these changes were prevented by all of the drug candidates. In addition, other changes were only affected by one of the drug candidates suggesting that these compound-specific changes might be able to provide a specific fingerprint for drug efficacy in future studies.

Acknowledgments: This work was supported by the Salk Institute Pioneer Fund Postdoctoral Scholar Award and the Salk Nomis Fellowship Award to AC and grants from the Alzheimer's Association, Burns Foundation and National Institutes of Health (grant numbers RO1AG046153, RO1AG035055 and R42AI104034) to PM and DS.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Profiling and identifying compounds from the aquatic fern *Azolla*: Why?

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ABSTRACT

The search for novel compounds or new sources of bioactive compounds from plants are normally made in angiosperms not in ferns and so their potential is not fully investigated. The genus *Azolla* is composed by seven species that is unique since it is the only fern that harbours a permanent symbiosis with the cyanobacterium *Anabaena azollae*. The research of their natural compounds is spread in time and possible bioactivity is sparse. These ferns have several compounds such as fatty acids [1], phenols as luteolinidin, caffeic acid, aesculetin, chlorogenic acid [2], α-asarone and isoeugenol [3], terpenoids such as lupeol, cholesterol [2], lycoxanthin, α-sitosterol [3], cycloartenol, campesterol, sitosterol [4], volatiles [5] and much more. Despite the scarce data on this symbiosis, it has been indicated as having medicinal potential to cure sore throat and cough [6]. Further, the lipophilic extract had activity against *P. expansum*, while the hydrophilic extract presented activity against A. vitis and the crustacean A. salina [7]. Also, the methanolic extract of *A. microphylla* has antimicrobial activity against *Xanthomonas* sp. [8]. In a wide survey with six *Azolla* species, [9] establish that organic extracts of *A. caroliniana*, *A. rubra* and *A. filiculoides* inhibited the growth of *B. subtilis* whereas *A. caroliniana* and *A. microphylla* extracts inhibited the growth of *S. aureus*. Hence, this ferns seems to have the potential to be used as antibacterial, but nothing is known about the compounds or compounds that induce the bioactivities.

Acknowledgments: The European Social Funding (FSE) under the Human Potential Operational Program (POPH) of National Strategic Reference Board (QREN) supported the fellowship SFRH/BPD/44459/2008 to Ana L. Pereira.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Effect of adaptive changes in lipids on conformation of OmpF porin from *Yersinia pseudotuberculosis*

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ABSTRACT

Bacteria contact with the environment through the cell membrane lipid matrix. Compensatory changes in the fatty acid (FA) composition and the head groups of membrane lipids during adaptation provide unique dynamic and structural properties of the membrane that are necessary to support the ability of microorganims to live in the new conditions of existence. Probably, the same processes can influence resistance of bacteria to antibiotics and immune system of the host organism. It is assumed that lipid environment provides optimal conformational state of membrane proteins in the certain conditions. Therefore, changes in the physico-chemical properties of lipids should correlate with their effects on conformation, and therefore, on functions of membrane proteins. Thus, the understanding of the dynamic processes in membrane proteins in their native hydrophobic environment allows for a fresh look at their functioning. OmpF porin (YOmpF) is the dominant protein of the outer membrane (OM) of *Yersinia pseudotuberculosis* which performs an important role in the exchange processes between the cell and the environment. It is known also that porins are the channels for penetration of some antibiotics into the bacterial cell, and hence responsible for development of resistance to these drugs. Therefore, our work was aimed to study the influence of adaptive changes in lipids of Y. pseudotuberculosis on conformation of YOmpF, as well as establishing their role in the resistance of bacteria to antibiotic ampicillin. It was shown that stress predominantly induces accumulation of unsaturated form of lysophosphatidylethanolamine (LPE) which unlikely saturated form increases thermal stability of YOmpF compacting protein monomers [1]. Mostly LPE is localized in OM of *Y. pseudotuberculosis*, where YompF is situated too [2]. DSC and intrinsic fluorescence were shown that total lipids from *Y. pseudotuberculosis* enriched with LPE increased thermal stability of the protein, associated with the compaction of its hydrophobic region, to a greater extent than the total lipids with a low content of LFE [3]. An adaptive changes in the level of LPE correlates with the sensitivity of bacteria to β-lactam antibiotic ampicillin. So, the accumulation of LPE in membranes is the part of adaptive response of bacteria to stress.

Acknowledgments: This work was supported by Russian Science Foundation Grant 15-15-00035**.**

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Metabolite profiling of rice volatile compounds from Portuguese varieties

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Available Online: 2 October 2017

ABSTRACT

The identification of metabolite profiling of rice volatile compounds affecting flavour and taste and can be used in breeding programs to produce locally adapted varieties that answer consumer preferences.

Six rice genotypes, including two commercial varieties, Ariete (japonica), Sprint (indica); two new Portuguese varieties, Maçarico (OP1001), Ceres (OP1203) and two advanced lines OP1109, OP140 were selected to analyse metabolite profiles and assess the stability of detected associations in 6 environments: 3 different locations (Alcácer do Sal-AS, Salvaterra de Magos-SM and Bico da Barca-BB) and harvested in two years. Volatile compounds were measured by GCxGC-TOF-MS, and multivariate analysis was conducted. The resulting data were represented through Principal Components Analysis (PCA).

The volatile compounds from the rice grains led to clustering based on the growing location, which is most unusual. Generally, the volatile compounds from grains lead to clustering based on genotype. This suggests significant genotype by environment interactions. While there were 220 compounds in the volatile headspace of the rice varieties, only a few show discrimination that explains the clustering. The most discriminating compounds are mainly alkanes and alcohols responsible for separating the samples grown at AS (Figure 1, Group 1) than those grown at SM and BB

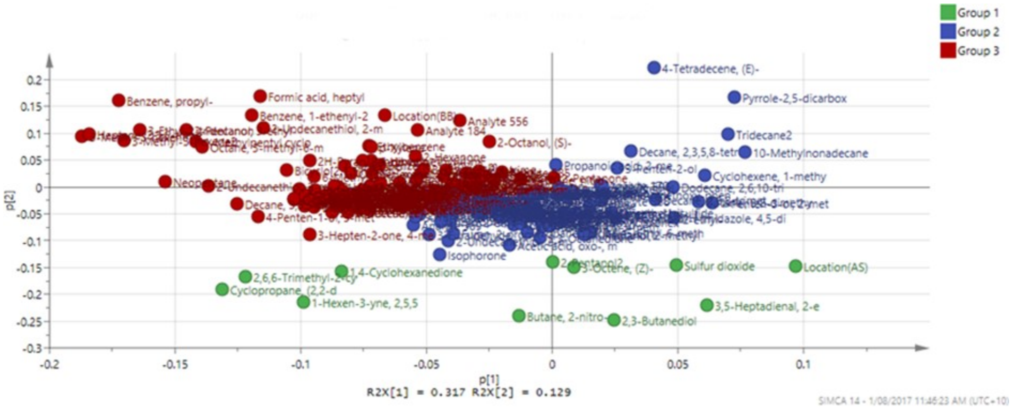

Figure 1 | Volatile compounds obtained from six rice genotypes harvested in 6 environments.

Acknowledgments: Funding from the Portuguese Fundação para a Ciência e Tecnologia (FCT) under the grant agreement RECI/AGR-TEC/0285/2012, BEST-RICE-4-LIFE.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Statistical Inference of Gene Regulatory Network from Gene Expression Profiles

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Available Online: 2 October 20177

ABSTRACT

he rapid advances in experimental technologies means that we can obtain the several types of omics information as profiling data in living cells. Those profiling data is the results of some of cell activity for surviving or adapting under several conditions, and we can uncover the cell activity mechanism via disclosing these profile data. Among the several types of profile data, gene expression profiles are obtained by complex functional transcriptional regulations in cells. To clarify the mechanism of those complex regulations, inference of gene regulatory network is one of the useful approach. Actually, various algorithms including Boolean and Bayesian networks, have been developed to infer complex gene networks [1][2]. In our previous investigation, we developed an approach based on graphical Gaussian modeling (GGM) combined with hierarchical clustering to infer the huge network among all of the genes [3]. Although all of these approaches are feasible for establishing relationships among genes, it is difficult to reveal the critical interactions between genes and the other cellular components, owing to the insufficient information about the other cellular components in the gene expression profiles. Since the underlying mechanism for transcriptional regulation in living cells, regulations of genes and the effects from other cellular components should be considered. Estimation of regulatory networks among genes and the other cellular components is absolutely essential to uncover the mechanism of gene expression control, and an alternative approach is needed.

We developed a statistical approach to obtain better insights of the transcriptional regulatory mechanism from gene expression profiles [4]. Our approach based on Structural Equation Modeling (SEM) in combination with factor analysis and new algorithms for initial model assumption and model optimization. SEM can include the latent variables within the constructed model and infer the relationships among the latent and observed variables, as a network model. We improved a method for construction of initial models for SEM calculation, and applied our approach to estimate the regulatory network for several types of gene transcriptional controls. In this new approach, we combined cross-correlation and partial correlation to summarize the temporal information and to extract the direct interactions from gene expression profiles. This approach allowed us to reconstruct a model of transcriptional regulation that involves protein-DNA interactions and the RNA division effects from maternal cell to daughter cells from only the gene expression profiles, in the absence of protein information. In this presentation, we'll show you the details of SEM approach for detecting the causality between genes and other cellular components

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Next generation toxicology risk-assessment applied to novel nicotine delivery products

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ABSTRACT

In the past decade, novel nicotine delivery devices (e-cigarettes, heated tobacco products) have emerged as a substitute for cigarette smoking with the potential to be significantly less harmful than combustible products [1], yet, epidemiological studies are lacking. Due to intrinsic limitations of traditional toxicology testing, there has been a shift towards holistic alternative systems biology approaches. The NRC's "Toxicity Testing in the 21st Century" [2] outlines the utility of such approaches using human tissues, high throughput profiling screens and omics to dissect the cellular-response networks to support the mechanistic understanding of toxicity pathways. We have developed a systems toxicology strategy to assess novel nicotine delivery products in vitro and in clinical samples. In this presentation, we outline how RNA-seq toxicogenomics is used in combination with downstream causal reasoning to assess the effect of e-cigarette aerosols *in vitro* on a reconstituted human 3D airway tissue [3]. We further describe how a multiplatform metabolomics/lipidomics/lipoprotein/miRNA profiling method was applied to map biological changes in the serum of smokers [4]. Finally, we will place those results in the context of building adverse outcome pathways (AOPs) [5] relevant to tobacco-related diseases that can support the assessment of novel nicotine products.

Acknowledgments: The authors are grateful to Fios Genomics and Metabometrix Ltd for their support with the RNA-seq and metabolomics data analysis, respectively. This work was funded by British American Tobacco. Part of this work was contracted to Fios Genomics and Metabometrix Ltd.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Identification of new proteins associated with atherosclerotic plaque instability in the tandem stenosis mouse model

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ABSTRACT

Strokes and particularly myocardial infarction (MI), as the most frequent single cause of death, are typically caused by abrupt rupture of atherosclerotic plaques in arteries such as the coronary arteries. Despite major advances in the treatment of MI, improvements in mortality and morbidity are substantially limited by our inability to foresee plaque rupture and the resulting thrombotic vessel occlusion. We hypothesized that using our newly created tandem stenosis mouse model of plaque instability/rupture we will identify new proteins that might particípate in plaque progression or plaque vulnerability. Plaques samples from 120 ApoE deficient mice were collected after tandem stenosis surgery. Plaques were allocated into five segments according to the histological evaluation of plaque stability [1]. Segment I showed thin cap fibroatheromas as typically seen in human unstable plaque (Figure 1). Each segment was homogenized in lysis buffer and then LC-MS/MS was performed. Data analyses were performed using Proteome Discoverer software (Thermo Scientific) and mouse Swiss-Prot database (Mus musculus, including 16.717 reviewed canonical entries). Following serial selection criteria, only the high confidence peptides were included for further analysis. We identified multiple protein targets that had a strong correlation with plaque instability and intraplaque hemorrhage. These newly identified proteins were validated by immunohistochemistry in tandem stenosis mouse plaques as well as in human carotid endarterectomy plaques. Finally, these proteins belong into diverse functional categories, including cell adhesion, protein metabolic processes and celular component morphogenesis. The gene ontology shows an association of the integrin signalling pathway and the cytoskeletal regulation pathway with plaque instability. Overall, proteomic analysis of unstable atherosclerotic plaques in comparison to stable atherosclerotic plaques holds promise to identify proteins involved in plaque instability and thus to define potential novel targets for plaque stabilization.

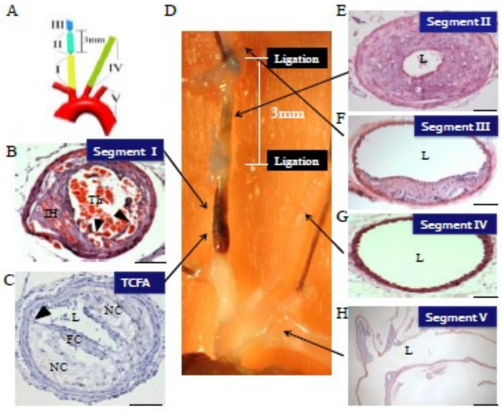

Figure 1 | (A) Schematic drawing of each segment. (B, C) Segment I showed thin cap fibroatheroma and intraplaque hemorrhage (D) Gross anatomy of TS model (E) Segment II showed positive outward vascular remodelling. (F, H) Segment III and V showed stable atherosclerotic plaques containing thick caps and small necotic cores. (G) Segment IV represented healthy vessel. L: Lumen; FC:Fibrous Cap; NC:Necrotic Core; Th:Thrombus; IH: Intraplaque hemorrhage.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Immunochemical characteristic of antigenic structure of OmpF porin from *Yersinia pseudotuberculosis*

Olga D. Novikova*, Olga Yu. Portnyagina, Valentina A. Khomenko, Marina P.Isaeva, Elena F. Solov'eva, Tamara F. Solov'eva

ABSTRACT

Many authors consider antigenic mimicry as the main reason of autoimmune diseases. The immunodominant antigens (namely non-specific porins) of bacteria of *Yersinia* species are proved to be similar to antigens of human organ tissues (including thyroid gland (TG) tissue) that are precondition for the development of autoimmune process in the organism infected. Outer membrane (OM) non-specific porins of Gram-negative bacteria belong to the β-structured integral membrane proteins. Structural elements of the porins corresponding to the outer loops connecting β-strands are shown to coincide with hydrophilic maxima and to form porin antigenic determinants. The present work is aimed to determine the role of external loops of recombinant OmpF porin from OM of *Yersinia pseudotuberculosis* (YpOmpF-r) in antigenic structure of the protein. Mutant porin monomers of YpOmpF-r with deletions of the loops L1, L4, L6 and L8 (hereinafter - del1, del4, del6 and del8) and specific antisera to full-sized and mutant porins studied were used for immunochemical characteristic of the protein. According ELISA results, the greatest number of B-epitopes was lost following the deletion of loops L1 and L4. Analysis of interaction between mutant porins and the pool serum of patients with Graves' disease and monoclonal antibodies (Abs) to hormone thyrotropin receptor (TSHR) showed that the porins without one of the outer loops (del1, d el4, del6, del8) bind to these antibodies differently (Figure A and B).

According the data of Figure A and B, del1 porin interacted very weakly with pool serum of patients with Graves' disease and did not practically interact with mAbs to TSHR. Thus, it can be assumed that outer loop L1 of YpOmpF-r comprises the region of amino acid (AA) sequence of the protein having a certain degree of homology with some immunodominant site(s) of TSHR molecule. However, a comparative analysis of the AA sequences of YpOmpF and the TSHR subunit A showed not only a sufficiently low degree of their overall homology, but also the lack of extended homologous regions in the primary structure of both proteins. Only some motifs containing two and three AA residues corresponding to fragments of the L1 sequence were found. In this regard, the most likely cause of cross-reactivity of YpOmpF and TSHR is the presence of the distinct segments of AA sequence with similar spatial organization at the level of the protein tertiary structure.

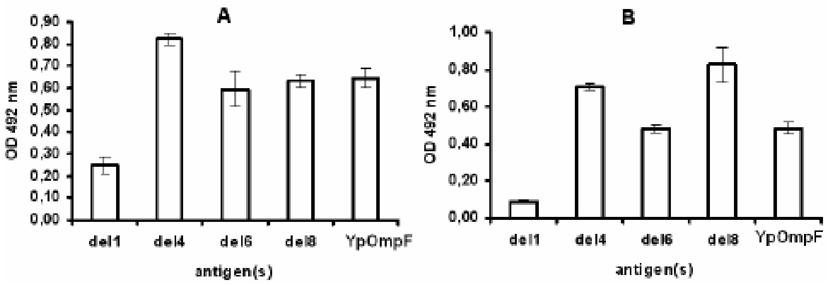

Figure 1 | ELISA analysis of interaction between deletion mutants of YpOmpF-r (del1, del4, del 6, del8) and full-sized YpOmpF-r with: (A) pool serum of patients with Graves' disease and (B) with mAbs to TSHR (dilution 1/500).

Acknowledgments: This work was partly supported by RFBR grant № 16-04-00424.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Untargeted profiling of phenylalanine derived metabolites in wheat ears during *Fusarium graminearum* infection by ^{13}C-labeling, LC-HRMS and custom data processing

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ABSTRACT

Fungi and their host plants have large arsenals of secondary metabolites, many of which have designated functions during pathogen-host interaction. For example, the fungus *Fusarium graminearum* produces deoxynivalenol (DON), which is an inhibitor of protein synthesis [1] and a toxic contaminant of food and feed [2], while wheat plants synthesize secondary metabolites to defend themselves against the pathogen (e.g. phenylpropanoids [3]).

To investigate the response of wheat plants to *Fusarium* infection, we have established an untargeted metabolomics workflow designed for detecting biotransformation products of uniformly isotopically labeled tracer compounds using LC-HRMS and an in-house developed data processing software. We have started from the aromatic amino acid phenylalanine, which is further synthesized into many different compound classes such as courmarins, hydroxycinnamic acid amides, and flavonoids. Although many compounds are already known, our workflow allowed probing these compounds in an untargeted manner using the highly specific isotopolog pattern of the ^{13}C-labeled phenylalanine units. In total, we have detected 144 phenylalanine-derived metabolites, of which around 70 could be annotated with previously known phenylpropanoids. We have then tested their abundance levels in DON- and mock- (water) treated samples and found that these metabolites clearly separated the two experimental groups in a multivariate statistical analysis. In a univariate comparison using t-tests (p-value $\leq$ 0.05; mean-fold-change $\geq$ 2) approximately 50% of all biotransformation products had increased levels in the DON-treated samples further suggesting their involvement in defense-related mechanisms of wheat against *Fusarium graminearum.*

Acknowledgments: This work was supported by the Austria Science Fund (projects SFB Fusarium 3706/3715 and T2/HT2 P26213) and the Government of Lower Austria (projects NovAlgo/NoBiTUM).
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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Interaction of OmpF porin from *Yersinia pseudotuberculosis* with antibodies to human thyroid-stimulating hormone receptor. Study *in vitro* and *in silico*

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ABSTRACT

Previously, it was shown that monomeric form of *Yersinia pseudotuberculosis* OmpF porin (YpOmpFm) could interact with the pool serum of patients with Graves' disease and with monoclonal antibodies (mAbs3B12) to thyroid-stimulating hormone receptor (TSHR). In present work, we carried out molecular modeling of the interaction of YpOmpFm antigen epitopes with a stimulating antibody (Abs, M22 Pdb ID 3g04) to TSHR. At the first stage of our study using different online resources and based on the primary and spatial structure of the YpOmpFm, a set of probable antigenic B-epitopes that satisfy the requirement of availability to the solvent was predicted. There were two small conformational epitopes: the first one was formed as a result of the convergence of the regions comprising two outer loops L4 (D158) and L6 (246NK247), as well as two β-strands, β11 (230ETQ232) and β13 (D276). The second region was formed with the participation of loop L2 (66EDS68, 70AGD72) and adjacent β-strands: β2 (36G, 38F) and β3 (W56). At the second stage of our study the participation of the amino acid (AA) residues forming the predicted B-epitopes of YpOmpFm in the interaction with Abs to TSHR was confirmed by molecular docking performed using MOE CCG® program (Fig. A). It should be noted that in the AA sequence of YpOmpFm and TSHR there are no extended homologous regions. However, for both proteins AA residues with similar properties and position on the binding surface in the interaction area with the Abs were revealed (Fig. B, C). According our results, E107, K209 and D232 of TSHR were overlapped with E66 K163 and D158 of the porin with binding energy contribution -11.1, -25.86 and -7.65 kcal/mol, respectively. Hydrophobic cluster F130, F153, I155 of TSHR was overlapped also with W56, Y58 and F38 of porin (total contribution -11.71 kcal/mol). Thus, a similarity of the spatial structures of YpOmpFm and TSHR that determines their cross-interaction as antigens has been established. Consequently, antibodies to porin may interact with TSHR inducing excessive stimulation of thyroid gland and thereby causing the autoimmune disorder with the symptoms of hyperthyroidism that is characteristic of Graves' disease.

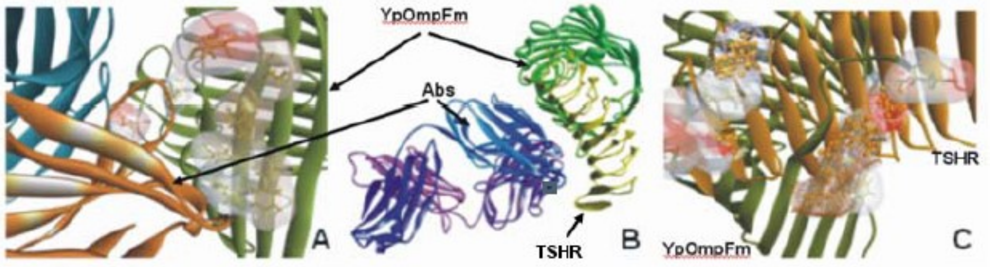

Figure 1 | Ribbon diagrams of: Abs to TSHR interaction with YpOmpFm B-epitopes (A); superposition of complexes Abs to TSHR with YpOmpFm and TSHR (B) and overlapped epitopts of YpOmpFm and TSHR (C).

Acknowledgments: this work was supported by RFBR grant № 16-04-00424 .
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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Development of a transcriptomic protocol combining bovine somatic cells and OpenArray® technology to trace the ab(use) of somatotropin in dairy cattle

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ABSTRACT

The administration of exogenous recombinant somatotropin (rbST) in dairy cattle is used to increase the milk yield. Since its approval in dairy production in 1993, the use of rbST has been controversial due it possible harmful effects in animal welfare and human health. While their use is approved in countries as United States of America, Mexico or Brazil, the European Union (EU) banned their use in 1999. In spite of this ban, in the year 2013 the illegal use of somatotropin was detected in Spain, making the authorities aware of the need of control the illegal use of this substance in the EU. For this purpose, the development of analytical methods to detect their fraudulent use is necessary in the EU. Analytical chemistry methods based on the direct detection of the banned substance as Liquid Cromatography Tandem Mass Spectrometry are the first option to detect their use [1]. However, some commercially available rbST has the same amino acid composition that the natural bST, rendering impossible their differentiation. Therefore it is of great importance to develop indirect methods that allows detecting the use of rbST in cattle [1]. In the last years the transcriptomic technology experiences a boom due to the development of RNA-seq, microarrays or High-Throughput Real-Time PCR. Real-Time PCR is considered the gold standard for quantification purposes, allowing the measurement of small differences between samples. Recently, transcriptomic has been used as a tool to detect the use of growth promotors in beef cattle [2]. However, these studies are focused in the use of target tissues as liver or muscle obtained *post-mortem*. In the case of rbST, it is key to control their misuse *in vivo*, during the period of lactation. Therefore, target samples have to be easy to collect, the method of collection should be non-invasive and it has to be economically viable. The goal of this study was to develop a transcriptomic protocol based in the combination of High-Throughput Real-Time PCR and milk somatic cell (MSCs) to detect the ab(use) of rbST. MSCs were collected from 6 treated and 3 control cows and different time points to analyze the expression of selected genes. To represent real conditions, cows were housed in a semi-intensive farm and administred with 500mg of rBST every two weak (Lactotropina®, Elanco®, Eli Lilly, México). The results showed that MSCs are an optimal alternative to isolate RNA with good quality using a simple protocol for their collection. The use of OpenArray® technology allowed the simultaneous analysis of 18 selected genes for 48 samples taken at different treatments points. With this technology it was possible to stablish a transcriptomic profile of treated and control cows that could be used to detect the illegal use of rBST in dairy cattle in EU.

Acknowledgments: Authors acknowledge the financial support of the Spanish Innovation Program "Programa *Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad*" (project AGL2014-58881-R).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

GC-TOF-MS Metabolite Profiling of Drought Tolerant *Quercus ilex*

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ABSTRACT

Holm oak (*Quercus ilex* L.) is the dominant tree species in natural forest ecosystems of the Western Mediterranean Basin. This species is well adapted to summer droughts but may not be able to cope with future increases in drought intensity, duration, and/or frequency as the climate becomes warmer and the water availability decreases [1]. In order to better understand the mechanisms underlying drought tolerance in *Q. ilex*, a metabolite profiling analysis was performed with gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) using leaves of two-year-old *Q. ilex* seedlings subjected to increasing drought severity. Leaf sampling was carried out at mild, moderate, severe and very severe water stress conditions. Primary metabolites were extracted and analysed using a well-established protocol for metabolite profiling described by [2]. A set of 31 primary metabolites were detected in *Q. ilex* leaves. Among these, amino acids and derivatives were the most abundant metabolites, followed by sugars and sugar-alcohols and organic acids. Mild water stress caused most sugars and sugar alcohols to increase, suggesting a role of these metabolites in stress signaling and osmoregulation. These metabolites continued to increase through moderate to very severe water stress conditions. At very severe water stress conditions most amino acids dramatically increased, especially γ-aminobutyric acid (GABA) and proline suggesting enhanced protection against oxidative damage. These results indicate that high drought tolerance of *Q. ilex* relies on early water stress signaling and osmoregulation by hexoses and polyols, and enhanced protection against oxidative damage by amino acids at severe water stress. *Q. ilex* has shown mechanisms of acclimation to drought, which can be useful for its persistence under a future drier climate.

Acknowledgments: The authors are thankful to Guillermo G. Gordaliza and Meng Li. A.M. Rodrigues acknowledges FCT for the PhD fellowship (PD/BD/114417/2016) and the ITQB NOVA International PhD Programme "Plants for Life" (PD/00035/2013). J. Rodríguez-Calcerrada acknowledges funding by the project OLMOS (AGL2012-35580). C. António acknowledges support from FCT Investigator Programme (IF/00376/2012/CP0165/CT0003), ITQB NOVA R&D GREEN-it 'Bioresources for sustainability' (UID/Multi/04551/2013), and LabMet metabolomics facility at CTBE (Campinas, Brazil) for GC-TOF-MS metabolite profiling services..

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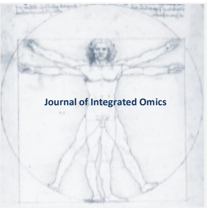

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Fattyacidomics and minerals: profiling the effects of recombinant bovine somatotropin on milk composition

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ABSTRACT

Growth hormone or somatotropin (ST) is a species-specific polypeptide hormone produced in the pituitary gland of vertebrates. When administered exogenously to dairy cattle, it has galactopoietic effects and is capable of increasing the milk yield. The commercial production of recombinant bovine ST (rbST) enabled large-scale applications in farms, enhancing significantly milk production. While it is banned in the European Union (EU), several countries permit the trade and use of recombinant somatotropins in animal husbandry. The literature reflects the existence of effective analytical methods to trace rbST presence in milk and other matices [1]. However, very little effort has been invested into defining the impact this treatment may have on the quality of the dairy products obtained. In this context, *profiling* and *omic* technologies offer a good opportunity to assess various components simultaneously in food [2]. This study is meant to profile the measurable effects or rbST on the nutritional properties of milk.

A group of nine cows was separated; 6 animals were treated every two weeks with a dose of Lactotropina® (i.e. 500 mg of rbST), for a period of 8 months, while the other 3 were used as controls. Milk samples (> 400 milk samples) were collected freshly, at different time points (first milking of the day). Fifty fatty acids were measured using GC-FID, gross composition was obtained by infrared spectroscopy in a certified Spanish laboratory (*Laboratorio Interprofesional Galego de Análise do Leite* – LIGAL) and minerals (Ca, P, K, Na, Mg) were measured by ICP-MS.

The implementation of univariate and multivariate statistics showed a tendency towards a less saturated fatty-acidome in the milk collected from animals treated with rbST, with higher concentrations of omega-6 and monounsaturated fatty acids. Conversely, the short chain fatty acids and various omega-3 were higher in controls. In addition, less calcium and protein content and more potassium was observed in milk from treated animals, in comparison to the control population. Thanks to this multi-component profiling of milk, a clear impact of somatotropin treatment on milk qualities was observed. The obtained results should be particularly interesting for those countries that permit the use of this hormone in dairy production.

Acknowledgments: This work is funded by the Spanish Innovation Program "*Programa Estatal de Investigación, Desarrollo e Innovación Orientada a los Retos de la Sociedad*" (project AGL2014-58881-R).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

New Protocols for NMR-based Metabolic Profiling

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ABSTRACT

NMR spectroscopy is extremely powerful for the identification and monitoring of individual metabolites in complex mixtures encountered in metabolomics.[1] It can be applied to a wide range of biological samples, such as urine, serum, tissue extracts, cell lines, etc. Protocols will be presented for optimal sample preparation to ensure reproducible, high quality NMR data.

In order to be able to monitor a maximal number of metabolites in these samples, we use 2D NMR experiments, including 13C-1H HSQC, 13C-1H HSQC-TOCSY, and homonuclear TOCSY. We have streamlined the analysis of these spectra by our COLMAR suite of web servers that permit the uploading of peak lists extracted from these spectra or the entire 2D data sets for query against the COLMAR spectral databases, which have been customized for the different types of 2D spectra. First, the HSQC query provides a rank-ordered list of metabolite candidates along with quantitative metrics. These hits are then validated using either one or both TOCSY-type experiments providing spin-connectivity information across the molecules. This workflow has been implemented in COLMARm (http://spin.ccic.ohio-state.edu/index.php/colmarm/index) alllowing the user to carry out this process in a computer-assisted, semi-automatic fashion (Figure 1). The identification step is followed by quantification of individual metabolites, which can be performed on a cohort of samples for profiling applications. Examples we will be provided for selected systems.

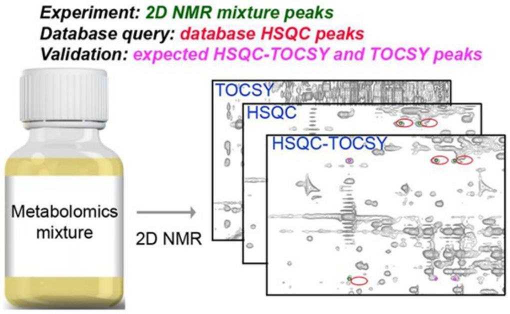

Figure 1 | Flow chart of the semi-automated COLMARm web server for metabolite identification in a complex mixture. COLMARm enables high-throughput metabolomics studies.

Acknowledgments: This work was supported by the National Institutes of Health.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

About the origin of the matrix mechanism and the genetic code

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Abstract

The matrix synthesis mechanism of transcription and translation can be originated in the defective zone of the crystalline lattice of co-crystallizing minerals: apatite, carbonate-apatite and calcite in mutually coordinated ratio, with the assistance of the gas-phase radicals and ions (NH_3, CH_4 and CO_2)[1]. This is the area of rhythmic changes in chemical composition, of isomorphic substitutions and vacancies and of occurrence of significant energetic fluctuations of thermal vibrations of atoms in the structure of this minerals. This led to the emergence of the growing center of the disorder and to appearance of the need to reset the energy potential in the form of stratified zones. All this is implemented in the structure of the emerging organo-mineral nucleoproteid complex. The ternary complex - DNA, RNA, protein - organized themselves in the area of apatite and carbonate-apatite according to the principle of stereospecific complementarity "spire in the spire". The double helix of DNA is a basis of this. Already at this stage all the necessary proteins for replication, transcription and translation related with their RNA were present, although the ribosomal apparatus has not been yet formed. The transition from a defect-free region in the apatite crystal to the occurrence of the disorder was realized in sizes and specificity of the genes in the DNA in the form of a gradual transition from the satellite zone (uninformative part of the DNA in eukaryotes) to the multiple repeated zone of the pre-tDNK, the moderately repeated zone of the pre-rDNA and the unique zone of genes of the pre-mDNK. The RNA-protein complex formed in the area of carbonate-apatite complementary to these zones. Each pre-tRNA in the crystal matrix interacted with its protein (aminoacyl-tRNA synthetase in future) stereospecifically. Each this protein already had amino acid and ATP in its composition. Each pre-rRNA also was associated with its proteins which were much due to the larger length of the fragments compared with the pre-tRNA and further which will be part of a ribosomes. Other proteins complementary associated with the first DNA- and RNA-associated proteins followed them further. Each pre-mRNA also had its own complementarily bound proteins. In the second stage, at occurrence water, crystal organomineral complex passed into the liquid-crystalline metastable state which could be disrupted by the change in ion concentration, by the appearance of protocells and by the matrix mechanisms starts. DNA is a virtual carrier of the genetic information. The true carrier of such information is the aminoacyl-tRNA-synthetase (AA-tRNA-synthetase) which stereospecifically complementarily associated with its pre-tRNA, an amino acid and ATP and determines not only anticodon structure in pre-tRNA (there may be up to the six of different these) but also the amino acid for its pre-tRNA which will be presented to the codon of DNA. Various pre-tRNA structure for the same amino acids acquire a degenerate anticodon via its synthetase via the compensatory loop in tRNA. In the unit cell of apatite structure the three base pairs (purin-pyrimidine) are formed at a height of 0, 50, 100 (the area of CaI), wherein the third pair is always at the border of cells. In our opinion, this area of increased energetic fluctuations is the cause of the genetic code degeneracy.

Acknowledgments: This work was supported by Russian Science Foundation (Grant 14-50-00034).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Regarding Core Issues of the Origin of Life: About protocells synthesis and biological asymmetry

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Abstract

The author discusses some principal problems of biochemistry and molecular biology concerning the origin of life on the Earth. It is based on his theory about the origin of protocells of pro- and eukaryotic types with the assistance of gas-phase elements (NH_3, CH_4 and CO_2), apatite matrix and its co-crystallizing minerals (carbonate apatite, calcite, mica) in mutually coordinated ratio [1]. This theory describes in detail the possibilities of synthesis of purine-pyrimidine bases, DNA chains, RNA chains and nucleoproteid complexes; the formation of transcription–translation apparatus and matrix mechanism, tRNA and rRNA; the appearance of the first proteins in the ribosomes; the reason of code degeneracy according to the third nucleotide; and also considers how the structural asymmetry of molecules could arise and other fundamental questions of organization and of functioning of living cells.

Synthesis of protocells without violating the second law of thermodynamics. Cells forming through the transition from the crystalline matrix to the liquid-crystalline organo-mineral matrix is accompanied by a loss of the crystal energy by changing the bond types to hydrogen bonds and the emergence of stereospecific complementarity. Hydrogen bonds are much weaker than covalent one and coordination bonds of the crystal lattice. As a result, a rigid system of bonds of the mineral crystal lattice becomes modified. In general, the entropy of the new complex is much larger than the entropy of the crystal lattice.

The problem of biological asymmetry. Molecular asymmetry (chirality – right- or left-handedness) is one of the fundamental characteristics of living matter. The question about L-amino acids and D-sugars remains unanswered rather than about their racemic mixture [2]. In our opinion, the cause of the chirality is associated with an external asymmetrical (unidirectional from the center of the Earth to its surface) influence of temperature on forming of the complex chiral minerals and on structures of future protocells respectively. "When certain causes produce certain effects, the symmetry elements of the reasons should be manifested in the consequences caused by them" [3]. Synthesis of nucleic acids and proteins on crystals proceeded unidirectionally (upward) simultaneously by the principle "spire in the spire". This virtually excluded the possibility of racemic mixter forming, but did not prevent the emergence of the right or left helix. Apparently this determined structural asymmetry of monomeric units of nucleic acids and proteins.

Acknowledgments: This work was supported by Russian Science Foundation (Grant 14-50-00034).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Evaluation of a probable regulatory network between CYP1A1- CYP1A2 fragment and AHR on Coffee Consumption

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ABSTRACT

A previous pilot study examined if the genetic variability of caffeine metabolism could influence coffee consumption [1]. CYP1A2 is encoded by a gene located at 15q24 and presents polymorphism that can determine a decrease in the enzyme inducibility. Carriers of variant CYP1A2*1F allele are slow caffeine metabolizers, whereas individuals who are homozygous for CYP1A2*1A allele are fast metabolizers [2, 3]. Genomic-wide association studies (GWAS) of coffee drinking suggest a strong association with CYP1A1/CYP1A2 and AHR genes. According with their findings [4, 5], an increased intake of caffeine was associated with having a T-allele for CYP1A1-CYP1A2-rs2472297. They also found that another intergenic loci at 7p21 that corresponds to aryl hydrocarbon receptor (AHR), has a regulatory role in basal and substrate-induced expression of CYP1A1 and CYP1A2. The objective of the study is to examine if there is a relationship between coffee consumption and CYP1A1, CYP1A2 and AHR genotypes in the population of our previous pilot study. Our hypothesis is that the wide variability seen in caffeine levels between fast CYP1A2 metabolizers might have a correlation with changes in the genotype of the AHR and a regulatory region between the genes CYP1A1 and CYP1A2.

In our previous pilot study, we found that 8 out of 11 healthy volunteers presented a fast metabolizer phenotype and showed a large variability in their caffeine levels (0-0.67 mg/L) The objective of the present study is to examine if there is a relationship between coffee and caffeine consumption and CYP1A1/ CYP1A2 and AHR genotypes in the same population.

Acknowledgments: The authors acknowledge the fundamental support from Mrs. Valerie Yaughn, Director of the Library at South University, Savannah Campus in helping to find peer-reviewed papers that were of interest. Dr. Darcy Lima passed away last July 2015 and I wanted to acknowledge his infinite encouragement to write this and many other papers, book chapters and books and as research partner and enthusiast. He will be forever remembered.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Mass spectrometry-based metabolomics as a tool to study *Casuarina glauca* salt stress tolerance

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ABSTRACT

Casuarina glauca is a model actinorhizal plant characterized by its ability to establish symbiosis with nitrogen-fixing Frankia bacteria. This plant species grows naturally in coastal zones and is able to thrive under extreme salinity environments. *C. glauca* tolerance to high salinity is associated to biochemical and physiological adjustments such as low tissue dehydration, osmotic adjustments, and high membrane integrity. Mass spectrometry (MS)-based plant metabolomics has emerged as a powerful tool to address biological questions related to plant environment and agriculture. To date, there is almost no information on the *C. glauca* metabolome. In this study, a modern metabolomics approach that combines two MS-based analytical platforms, namely LC-QIT-MSn target analysis and GC-TOF-MS metabolite profiling, is being applied to study the impact of salt stress in nodulated and non-nodulated *C. glauca* plants. Our most recent results agree with those previously obtained from morpho-physiological analysis, and provide new knowledge on the primary metabolome of *C. glauca*, its symbiosis with *Frankia Thr*, and its metabolic readjustments under increasing salt concentrations. Furthermore, the divergent metabolite responses particularly found in the amino acid metabolism suggest root and root-nodule specific metabolite responses, and support the fact that from 200 mM NaCl upwards, symbiosis was turned off. Based on these results, a second independent biological experiment is currently ongoing to assess, at the physiological and metabolite levels, the performance of non-nodulated C. glauca plants under a combined salt and heat stress.

Acknowledgments: This work was supported by Fundação para a Ciência e a Tecnologia (FCT) through the project PTDC/AGR-FOR/4218/2012, the FCT Investigator Programme (contract IF/00376/2012/CP0165/CT0003 C. António), and the research units UID/GEO/04035/2013 (GeoBioTec), UID/AGR/04129/2013 (LEAF), and UID/Multi/04551/2013 (ITQB NOVA R&D unit GreenIT). T.F. Jorge further acknowledges FCT (PD/BD/113475/2015) and the ITQB NOVA International PhD Programme 'Plants for Life' (PD/00035/2013) for the PhD grant.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

New human plasma type 2 diabetes mellitus biomarkers

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Available Online: 2 October 2017

ABSTRACT

One of the central problems of modern medicine is the development of new approaches for diagnosis, treatment and monitoring of diabetes mellitus and its complications. Glycation is a post-translational modification formed by interaction of reducing sugars (e.g. glucose) with protein amino groups, yielding early glycation products (Amadori compounds). The latter undergo oxidative degradation, accompanied by formation of advanced glycation end products (AGEs). The abundance of glycation products in human plasma strongly correlates with hyperglycemia, characteristic for type 2 diabetes mellitus (T2DM). Currently, glycated proteins (hemoglobin and albumin) are routinely employed as clinical T2DM markers. However, these species show low sensitivity to short-term changes in blood glucose concentration.

In contrast, individual glycation sites in blood plasma proteins with different half-life time might be considered as alternative T2DM markers [1]. To prove this concept, the patterns of potential biomarkers were compared in cohorts (n = 20) of T2DM patients (women 45-75 years old with the HbA1C levels of 7.5-10%, not undergoing insulin therapy and not smoking) and age-matched normoglycemic controls. Individual glycation sites were analyzed by the LC-based bottom-up proteomic approach, including protein digestion, enrichment of early glycation products by affinity chromatography on boronic acid (BAC) and solid phase extraction prior to RP-HPLC-QqTOF-MS. Sequence assignment of differentially abundant glycated peptides relied on RP-HPLC-ESI-LIT-Orbitrap-MS/MS, whereas their biomarker potential was addressed by label-free relative quantification. Statistical analysis relied on the Mann-Whitney test and linear discriminant analysis (LDA). Totally, 51 differentially glycated protein lysine residues were identified, among them 42 individual glycation sites worked as biomarkers of type 2 diabetes. Thereby 32 glycated peptides were characterized as new potential biomarkers of T2DM [1]. The LDA approach showed variable set containing 12 biomarkers distinguished T2DM patients from the normoglycemic controls that might be useful for diabetes prediction, early diabetes diagnostic, therapy control, and patient stratification according to the concept of personalized medicine.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Unveiling the role of human cardiac stem cells in acute myocardial infarction

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Abstract

After an Acute Myocardial Infarction (AMI), Ischemia-Reperfusion (I/R) injury is characterized by a substantial decrease in the number of cardiomyocytes (CMs). Human myocardium harbors a population of endogenous cardiac stem cells (hCSCs) that is activated upon I/R injury, contributing to myocardial repair through the establishment of an auto/paracrine crosstalk between hCSCs and CMs in stress.

Cardiomyocytes response to I/R has been extensively studied, while CSC role in myocardial I/R is still lacking characterization. In this study, we set up an in vitro human cellular model of myocardial I/R injury using donor derived hCSCs and CMs differentiated from human induced pluripotent stem cells (hiPSC-CMs) to further decipher the action mechanisms of hCSCs upon injury.

Monocultures and co-cultures of hCSCs and hiPSC-CMs were established. Ischemia was mimicked by culturing the cells at 0% O2 in Ischemia Mimetic Solution. In the reperfusion step, cells were placed back in their physiological conditions of oxygen (3%) and nutrients. The effect of I/R injury in hCSCs was accessed by total proteome analysis at different time points using nanoLC-MS (*Eksigent LC4500* & *TripleTOF 6600*) and evaluated by IPA software. Growth factor secretion, cell viability, as well as hCSC proliferation were also evaluated.

Important features of I/R injury were successfully captured, namely CM viability loss, hCSC proliferation activation upon insult and the protective role of hCSCs on hiPSC-CMs. The culture readouts obtained together with the proteins identified in the different time points of the insult, allowed us to propose new possible players on hCSC regeneration response upon injury including activation of pathways related with cell proliferation, paracrine signaling, stress response and metabolism.

The human cellular model established in this work will allow further profiling and understanding on the molecular landscape of AMI, namely regarding hCSC regenerative response. This will potentiate the development of novel cell-and/or molecular-based therapies for myocardium regeneration.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Keratinolytic activity of Bacillus subtilis AMR wilde type and mutants

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Available Online: 2 October 2017

ABSTRACT

Keratin is a insoluble protein found in feathers, wool, horns and hair. They represent important residues in industry. Keratinases have been described to be produced by different microorganisms and can have application in food, animal feed, leather, detergent, pharmaceutical, cosmetic and textile industries.

The *Bacillus* gender has been used in different biotechnology applications, since most species are considered as GRAS (Generally recognized as safe), been used as probiotic. Previous study from our group selected bacilli with high keratinolytic activity. One of these strains, *Bacillus subtlis* AMR was isolated from agro-industrial waste of poultry industry.

In this work, the strain *Bacillus subtilis* AMR was mutagenized to improve the keratinolytic activity. The cells were incubated with ethyl methanosulfonate (EMS) at a final concentration of 1%. Gelatinolytic and keratinolytic mutants were selected on gelatin and keratin agar and compared with the activity of the wilde type.

The selected mutants were evaluated by zymography. The results showed that the mutants had a higher activity than the wilde type strain. Detected bands on proteic substrates maintained the same molecular weight only showing variation in the intensity. However, some differences were observed in some peptidases of these mutants. Peptidase activity was inhibited by PMSF and EDTA and were considered to belong to serine peptidase group. Analysis of the degradation products by HPLC showed few differences in the profiles. These mutants and its peptidases can have applications in improving the nutritional value of animal feed, cosmetics, food, textile and leather industries. As a consequence, the degradation of poultry industries residues can reduce the environmental impact of those industries, generating a more clean production.

Acknowledgments: We would like to thank to CNPq and FAPERJ for the financial support .

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Using metabolic approach to uncover plant-bacteria interactions that affect antibiotic production

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ABSTRACT

Plant roots harbor many bacterial species that are in constant competition over space and nutrients, and are frequently found to exist as micro-colonies or biofilms. Both the Gram-positive *Bacillus subtilis* and Gram-negative c*ompetitors* can form biofilms on plant roots, presenting an intriguing model system to explore interspecies interaction between biofilms. During the interaction, *B. subtilis* advances towards its competitors while activating antibiotic synthesis and mining dead cell debris from the competing colony. Following this engulfment, cell-contact on the solid surface promoted engulfment and killing of the competitors biofilm. Our overall results indicate a mechanism that allows biofilms of *B. subtilis* to overcome biofilms formed by Gram-negative bacteria during colonization of plants. Strikingly, the plant host produces secondary metabolites that influences the efficiency of the killing by affecting the synthesis of bacterial antibiotics. Using a metabolome screening, we are now unravelling plant secondary metabolites that specifically activate the synthesis of antibiotics in the rhizosphere community. These results suggest that the composition of the plant microbiome can be orchestrated by higher-order interactions where the host directly regulates bacterial antibiotic production.

Acknowledgments: This research was supported by the ISF I-CORE grant 152/1, Mr. and Mrs. Dan Kane, Ms. Lois Rosen, by the Larson Charitable Foundation, by Ruth and Herman Albert Scholars Program for New Scientists, by the Ilse Katz Institute for Materials Sciences and Magnetic Resonance Research grant, by the Ministry of Health grant 712376 for alternative research methods, and by the France-Israel Cooperation - Maimonide-Israel Research Program grant 3-13021, and by the Israeli Science Foundation (No. 119/16). IKG is a recipient of the Rowland and Sylvia Career Development Chair.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Epigenetic variability among saffron crocus (*Crocus sativus L.*) accessions characterized by different phenotypes

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Available Online: 2 October 2017

ABSTRACT

Saffron (*Crocus sativus L.*) is a sterile triploid (2n=3x=24), initially assumed to be of autotriploid origin, although a growing number of evidences support alloploidy as the most probable mechanism to have occurred. The crop vegetatively multiplies year by year by means of corms. Corm multiplication does not generate genome variations with the exception of some spontaneous mutations that in a triploid saffron population are not easily detectable. At the present time, the real level of genetic variability inside saffron is still debated and in literature it is possible to recover contradictory articles providing contrasting results about if the species is monomorphic or not. In a preliminary characterisation of 50 saffron accessions of the WSCC (World Saffron and Crocus Collection, located in the Bank of Plant Germplasm of Cuenca), characters related to phenology (date of sprouting and flowering, duration of flowering), floral morphology (length and width of tepals, and length of stamen filaments and anthers) and saffron production (percentage of flowering corms, number of flowers per corm, saffron spice weight per flower) were measured and a big variation detected. This raises the question about the origin of such variability, and, considering that gene expression can be influenced both by genetic and epigenetic changes, epigenetic variation could be a possible origin of the alternative phenotypes. In order to have a deeper insight in the epigenetic of saffron, the present study was devoted to the analysis of the cytosine methylation among saffron accessions with different geographic origin and cultivated for at least three consecutive years in the same conditions inside the saffron "CrocusBank" collection. The analysis of the methylation was carried out by using the High C+G Patch (HCGP) Filtration method coupled with high throughput sequencing. The accessions have been selected based on geographic origin, different phenotypes, and different agronomic characters and were characterized by high or low saffron production, early and late flowering time. The presence of high epigenetic variability in DNA regions associated with gene expression was detected. Finally, in order to gain information on the stability along the years of the epigenetic in a vegetatively propagated plant, saffron epigenetics of 17 different accessions stored in the "CrocusBank" collection was analysed in 4 consecutive years from 2013 to 2016. Each accession, despite the cultivation in proximity in the same field and despite the presence of intra- and inter-accession variability, tended to maintain a proper epigenotype clearly different from the other accessions.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA SYMPOSIUM ON PROFILING (ISPROF 2017)

Understanding the importance of KIX domain proteins in different systems

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ABSTRACT

The KIX domain, important for protein-protein interaction, was first discovered as a part of the large multidomain transcriptional activator histone acetyltransferase p300/CBP. Later on, this domain was identified in Mediator subunit MED15. We found that in CBP, disorder region following the KIX domain has evolved from Med15 KIX domain. In both of these proteins, the KIX domain has been shown to be a target of activation domains of diverse transcription activators and found to be essential in several specific gene-activation pathways in fungi and metazoans. However, not much is known about KIX domain proteins in plants. We made an attempt to characterize all the KIX domain proteins coded by *Arabidopsis* and rice genomes. Interestingly, KIX domain was found not only in p300/CBP- and MED15-like plant proteins as known earlier, but also in F-box containing proteins in rice and DNA helicase in *Arabidopsis*. These findings suggest new roles of KIX domain in ubiquitin mediated proteasomal degradation of protein and genome stability. In *Arabidopsis*, we have found more than twenty proteins interacting with the KIX domain of MED15. In rice, expression analysis revealed overlapping expression of *OsKIX_3, OsKIX_5* and *OsKIX_7* in seeds of different stages of development, suggesting their individual or combined role during rice seed development. Moreover, the association analysis using the genotyping data of 136 *in silico* mined SNP loci in 23 contrasting rice genotypes and their grain length-specific phenotypic information identified three non-synonymous SNP loci in these three rice genes showing strong association with long- and short-grain differentiation. Interestingly, these SNPs are located within KIX domain encoding genomic regions. It, thus, indicates the additional importance of novel SNP loci/alleles identified in KIX domain containing genes for determining seed size in rice.

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A proteogenomics approach for identification of molecular determinants of the oncosuppressive actions of Estrogen Receptor beta in breast cancer

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Abstract

The Estrogen Receptor beta (ERβ) is a member of the nuclear receptor superfamily of transcriptional regulators endowed with oncosuppressive activities, antagonizing estrogen-induced carcinogenesis and inhibiting growth and oncogenic functions in luminal-like breast cancers (BCs), where its expression correlates with a better prognosis of the disease. By systematically applying interaction proteomics coupled to mass spectrometry (MS) to characterize factors acting in concert with ERβ for regulation of BC cell activity [1], among >300 interacting proteins we identified Argonaute 2 (AGO2) as a novel partner of this receptor in human BC cells. ERβ-AGO2 association was confirmed 'in vitro' and 'in vivo' both in the nucleus and cytoplasm, and was found to be RNA-mediated. AGO2 is an RNA-binding protein acting as a key effector of RNA-silencing pathways, due to its direct involvement in microRNA maturation and activity, that are both controlled also by ERβ [2], able to modulate chromatin remodeling, gene transcription and RNA splicing. Functional genomics was applied to investigate the biological roles of the ERβ-AGO2 complex in luminal-like BC cells expressing human ERβ. ChIP-Seq analyses demonstrated co-association of AGO2 with ERβ in a large number of chromatin binding sites of the receptor, and total and nascent RNA-Seq in ERβ+ vs ERβ- cells, and before and after AGO2 knock-down in ERβ+ cells, revealed a widespread involvement of this factor in the well known effects of ERβ on gene transcription rate and RNA splicing [3]. Many genes directly targeted by the ERβ-AGO2 complex are involved, among others, in growth-inhibitory and oncosuppressive pathways, indicating that AGO2 plays an active part in the antioncogenic activity of ERβ. Moreover, RIP-Seq demonstrated involvement of the receptor in RISC loading via interaction with AGO2. These results demonstrate that AGO2 is a pleiotropic functional partner of ERβ in BC cells, indicating that both factors are endowed with multiple roles for control of BC cell functions.

Acknowledgments: Work supported by: AIRC (Grant IG-17426), Italian Ministry of Health (Grant GR-2011-02347781), CNR (Flagship Project InterOmics), Univ. Salerno (Grant FARB 2015-16) and EU (Elixir-Ita HPC@CINECA).

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(URINOMICS 2017)

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Targets in urinalysis for Wilson's disease

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Available Online: 2 October 2017

ABSTRACT

Urine is a commonly used specimen for analysis with clinical importance to detect and manage a wide range of disorders, prevalent and rare. Laboratory urine samples are classified by the type of collection or by the collection procedure – first and second morning urine, a random urine portion, 24-hour urine sample and etc. Results interpretations are useful clinical tools.

Harmonization in laboratory medicine requires deep knowledge of all steps in total testing process - from pre pre-analytical to post – examination. Some critical factors (proper time for sampling; sample type; sample container and volume; transport and storage with appropriate time and temperature) may serve as quality indicators. An effective urine diagnosing strategy should be based on standard procedures for collection, transport and analysis.

Underlined impact of urine analysis in Wilson's disease (WD) management is the reason for our special interest in this biological specimen – sample preparation before analysis, analytical determination of copper in urine, frequency of urine examinations to monitor the adequacy of chelating therapy, prevention of probable adverse effects as nephrotic syndrome, effective clinical laboratory approach as whole in this very complicated clinical situation.

Our experience defines urine as a delicate but stabile biological matrix for copper analysis. The stability is rather higher in the case of D-penicillamine administration (1000 mg/day) in comparison to urine control group for both studied temperature regimens (ambient 15-25°C and refrigeration 2-8°C). Plastic containers and tubes are pointedly suitable for sample collection and pre-analytical preparation with guarantee the lack of contamination. Flame atomic absorption is acceptable analytical method for copper urine analysis in concentration range Cu ≥ 0.23 μmol/L (Limit of detection). Monitoring of urine protein concentration in 24-hour sample is recommended at least every 6 months in WD patients on D-penicillamine to prevent eventual development of nephrotic syndrome - rare but severe adverse effect.

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Increased Phosphate excretion in critically ill children

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ABSTRACT

Objective: To determine the prevalence of hypophosphatemia in critically ill children; to determine the mechanism of hypophosphatemia in critically ill children.

Methods: Levels of Serum phosphate, phosphate intake, renal phosphate handling indices and blood gases were measured on days 1, 3, 7 and 10 of PICU stay. Hypophosphatemia was defined as any serum phosphorus <3.8 mg/dL for children younger than 2 years and <3.5 mg/dL for children 2 years or older. Renal phosphate loss was assessed using TmP/GFR.

Results: Prevalence of hypophosphatemia was 71.6% (95% CI: 64.6-78.6). Renal phosphate threshold was significantly lower on all the days in hypophosphatemic group compared to that of non-hypophosphatemic. No statistically significant difference in the amount of phosphate intake was seen in both the groups. (Table 1)

Conclusion: Hypophosphatemia is highly prevalent in critically ill children. Increased phosphate loss in urine is one of the mechanism responsible for hypophosphatemia in critically ill children.

Acknowledgments:

The authors are thankful to enrolled children and their parents, lab clinicians and on duty resident doctors.

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Table 1

Variables	**Hypophosphatemia**	**No hypophosphatemia**	**P value**
D1 Phosphate intake (mg/kg) D1 Calorie intake (Cal/kg)	0.8 ± 6.1 3.1 ± 2.1	0.5 ± 2.9 3.2 ± 2.4	0.86 0.50
D3 Phosphate intake (mg/kg) D3 Calorie intake (Cal/kg)	14.4 ± 12.4 8.1 ± 6.9	24.4 ± 16.8 9.1 ± 8.5	**0.04** 0.6
D7 Phosphate intake (mg/kg) D7 Calorie intake (Cal/kg)	25.9 ± 21.1 18.4 ± 12.7	33.7 ± 22.5 24.3 ± 16.5	0.26 0.14
D10 Phosphate intake (mg/kg) D10 Calorie intake (Cal/kg)	31.2 ± 22.1 29.4 ± 16.5	48.1 ± 28.5 35.3 ± 21.0	0.39 0.50
Serum pH, mean ± SD			
D1	7.33 ± 0.01	7.31 ± 0.01	0.16
D3	7.37 ± 0.01	7.35 ± 0.01	0.28
D7	7.38 ± 0.01	7.36 ± 0.01	0.32
D10	7.38 ± 0.01	7.37 ± 0.01	0.45
$TmPO_4$/GFR(mg/dL),median(IQR)			
D1	2.3 (1.8, 3.4) (n=89)	4.3 (3.4, 5.5) (n=30)	**<0.001**
D3	2.2 (1.5, 2.8) (n=78)	4.1 (3.2, 4.9) (n=20)	**<0.001**
D7	2.0 (1.2, 2.9) (n=49)	4.2 (2.8, 5.5) (n=11)	**<0.001**
D10	1.7 (1.2, 3.2) (n=35)	4.4 (3.4, 5.2) (n=9)	**<0.001**
PTH,pg/mL,median(IQR)			
D1 Serum PTH	31.4 (17.8, 54.9)	43.5 (18.2, 60.6)	0.20

IQR: Interquartile range SD: Standard deviation

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Towards early detection of pancreatic cancer in urine

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ABSTRACT

With >8,500 deaths in the UK, Pancreatic Ductal AdenoCarcinoma (PDAC) is currently the 5th leading cause of cancer-related death, but predicted to become the second by 2030 [1]. PDAC is almost always diagnosed at an advanced stage when curative surgery is no longer possible and patients die within 6-8 months. Development of a test for early detection of this malignancy would therefore highly likely have a huge impact on survival of PDAC patients. For the last several years we have been interrogating urine samples for proteins, miRNAs and recently volatile organic compounds in order to find the biomarkers that would be useful in early detection of this malignancy [2, 3, unpublished data]. Our panel of three protein biomarkers (REG1B, LYVE1 and TFF1) that shows promise in differentiating healthy, benign and cancer patients' groups [2] was recently further validated using additional urine samples confirming the previous results (Figure 1).

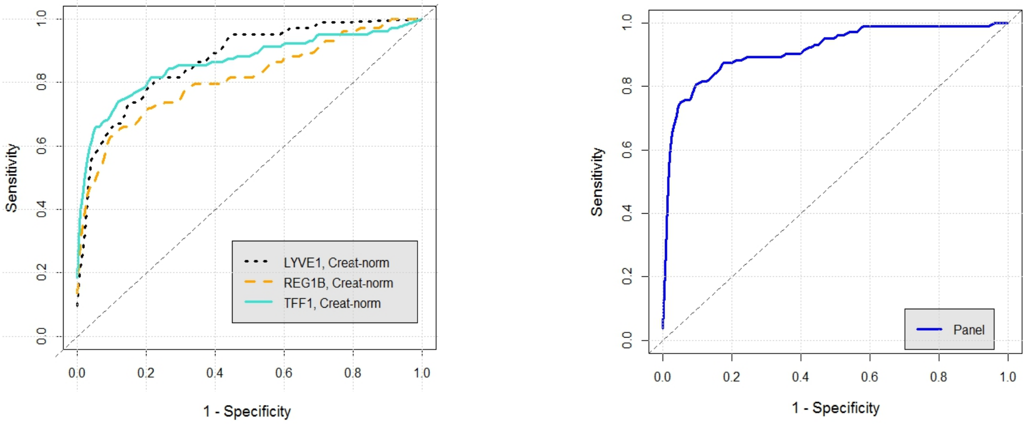

Figure 1 | Performance of urine biomarker panel in discriminating early patients with pancreatic cancer from healthy individuals: Receiver Operating Characteristic (ROC) curves of resectable stage I-II pancreatic cancer (n=103) versus healthy individuals (n=137) with AUC 0.92 (95% CI 0.88-0.95), sensitivity 80.6% (95% CI 72.8-87.4) and specificity 91.3 (95% CI 85.7-96.0).

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Acknowledgments:

Study was funded by Pancreatic Cancer Research Fund.
We thank the members of Pancreatic Cancer Research Bank for their continuous help .

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Rat detrusor muscle and vas deferens reactivity are negatively affected by diclofenac and indomethacin

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ABSTRACT

Introduction: Prostaglandins (PGs) and thromboxanes (TXAs) are locally acting hormones derived from arachidonic acid by the action of cyclooxygenase (COX) enzyme which exists in two distinctive isoforms, COX-1 and COX-2 (Smith, 1992). PGs have an important role in genitourinary tract function and disorders. Non-steroidal anti-inflammatory drugs are an important and efficacious class of drugs for the management of inflammatory conditions and they cause both beneficial and adverse effects via inhibition of the COX enzyme and subsequent inhibition of prostanoid synthesis (Crofford, 2013). Aim of the study: The study targets to screen the potential negative effect of COX inhibitors giving evidence about the differential sensitivity of urinary bladder, vas deferens and corpus cavernosum, and which tissue will be at lower doses. Contribution of knowledge in this field aims to enhance quality of life and reduce side effects and disease prevalence among different populations. Methods: Male Wistar rats (6-9 per group) were dissected and isolated bladder detrusor muscle, prostatic vas deferens and corpus cavernosum were used for electrophysiological organ bath chamber studies. COX-inhibitors were selected based on their COX1/COX2 selectivity (see Fig.1). All international and institutional guidelines for animal care and use were strictly followed. Results: Indomethacin, diclofenac and ketoprofen (20, 50, 100 μM) caused dose-dependent inhibition of both ACh and electric stimulation (ES)- induced contractility of detrusor muscle. EC50 of diclofenac was higher than that of indomethacin and ketoprofen. In vas deferens, indomethacin and diclofenac but not ketoprofen significantly shifted PE and ESresponse curves downwards at dose equal or higher than 50 μM. The basal contractile tone of corpus cavernosum was significantly increased by indomethacin and ketoprofen, an effect that was blocked in presence of TXA2 receptor blocker GR32191B. Indomethacin, diclofenac and ketoprofen significantly potentiated ES and ACh-induced relaxation or corpus cavernosum. SNP-induced relaxation was potentiated only in the presence of diclofenac. DFU (10^{-7}-10^{-5} M) did not show any significant effect on detrusor muscle and corpus cavernosum, however, it significantly potentiated PE-induced contractions of vas deferens. Conclusions: Diclofenac which possesses other COX- independent actions (such as LOX and TXA receptors inhibition and NO activation) showed the worst effect on bladder and vas deferens reactivity. Diclofenac and the highest selective COX-1 inhibitor, indomethacin are not recommended in bladder dysfunction and delayed ejaculatory problems. More COX-2 inhibitors seem to be devoid of this side effects but may precipitate premature ejaculation. Among the tissues tested, bladder detrusor muscle seems to be the most sensitive to the deleterious effect of nonselective anti-inflammatory drugs. COX-1 inhibition seems to enhance NO synthesis in corpus cavernosum but give upper hand to contractant PGs.

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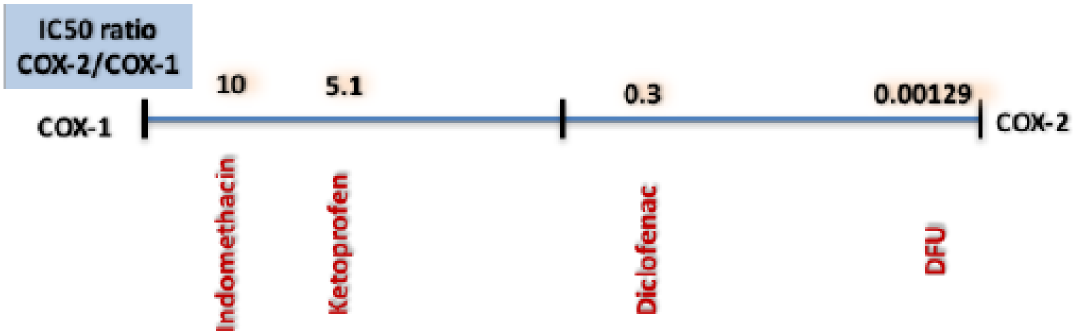

Figure 1 | (Different selectivity of chosen COX-inhibitors)

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Computer-based piRNA-mediated potential biomarker prediction in renal cell carcinoma

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Abstract

Renal Cell Carcinoma (RCCa) is the 9th most common type of cancer worldwide. Like other cancers, RCCa forms as a consequence of the accumulation of many genetic and epigenetic changes. Piwi interacting RNA (piRNA) is the largest class of small non-coding RNA molecules in animal cells. piRNA-protein complexes are associated with epigenetic and post-transcriptional gene silencing. Deregulation of some piRNAs has been observed in RCCa. Some genes (*VHL, ITPR1, PPAR, GPD1L, ABHD5, IMPDH2, CHDH, DRR1, PDHB and FHIT*) on the short arm of chromosome 3 are frequently deleted in RCCa cases [1]. Four piRNAs targeting at least four of these genes were determined using piRNAdb and piRNAQuest databases. The genes (*CALN1, ELAVL1, IL1RAPL1, PARK2, WWOX*) targeted by all of these piRNAs and showing the most potential competing endogenous RNA (ceRNA) activity were detected by piRNAdbdatabase (Figure 1). These piRNAs now regulate these five ceRNAs instead of deleted genes on chr.3 in RCCa. Previous studies showed *ELAVL1* is upregulated, and *PARK2* and *WWOX* are downregulated in RCCa so these findings make this in silico analysis logical [2-4]. Up to date, no association of *CALN1* and *IL1RAPL1* with RCCa in literature makes them as potential novel biomarkers in RCCa.

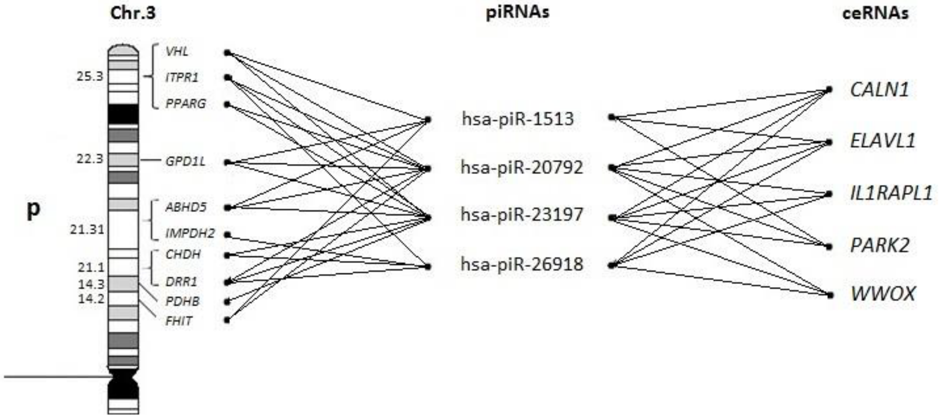

Figure 1 | In silico chromosome 3 deletions directed, piRNA-based ceRNA analysis in RCCa.

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Acknowledgments:

This work was supported Ordu University, Scientific Research Projects Coordination Unit.

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Three approaches for studying urinary cell free DNA in urological and non-urological cancers: integrity, copy number and mutations analyses

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ABSTRACT

The role of circulating cell free DNA in cancer has already been widely demonstrated, however, less is known about the role of urine cell free DNA (UcfDNA) [1]. UcfDNA can serve as a "liquid biopsy" for urological and non-urological tumors, as it carries information on DNA from both cells exfoliated in urine and circulation [2]. Recent studies showed that cancer-related mutations may be detectable in urine specimens, plasma and tissues samples from the same patients, for both urological and non-urological diseases [3;4], indicating that UcfDNA could provide a real-time picture of the disease without the need of invasive techniques. In the present oral presentation we will focus on three different molecular approaches to detect and monitor bladder, prostate, colon and non small cell lung (NSCL) cancers. In particular, we have tested UcfDNA integrity as a diagnostic marker for bladder cancer; *c-MYC* copy number variation for prostate cancer prognosis; *KRAS* and *BRAF* mutations for colon cancer and NSCLC in comparison with tissue and plasma results. Different DNA isolation procedures were tested starting from various urine volumes and a quality assessment of DNA was proposed using bioanalyzer High Sensitivity DNA kit. Real Time PCR approaches were used to analyze integrity of three different regions of interest (*c-MYC, BCAS1, HER2*), copy number variation of *c-MYC* gene, and *KRAS* and *BRAF* mutations. We generally obtained the same DNA quality and quantity for both cancer patients and healthy individuals. Urine cell free DNA integrity showed a sensitivity of 0.73 in detecting non muscle invasive bladder cancer patients, and a specificity of 0.83 in symptomatic patients [5]. *C-MYC* copy number gain was detected in about 25% of prostate cancer patients before prostatectomy and no copy number gain was detected in healthy individuals. Regarding mutations analysis we found a concordance of 70% between tissue, plasma and urine samples. We believe that UcfDNA will have an important role in cancer diagnosis, prognosis and monitoring for bladder, prostate and non-urological tumors. However, we observed a good Real Time PCR feasibility also for non cancer patients, suggesting that these approaches could be useful also for other disease types. The analysis of nucleic acids from different body fluids should be the real goal for a personalized medicine approach, allowing the detection of informative alterations for tracking disease course.

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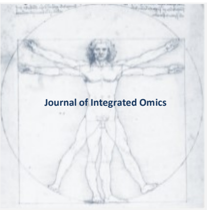

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Levels of urinary NGAL in resistant hypertension

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Available Online: 2 October 2017

ABSTRACT

Abstract. The purpose of our study was to investigate the importance of urinary neutrophil gelatinase-associated lipocalin (NGAL) in patients with resistant hypertension (RHp). A chemiluminescent microparticle immunoassay (CMIA) method became commercially available, using the automated platform ARCHITECT (Abbott Diagnostics) 1 for the measurement of NGAL in urine samples of RHp (33 with estimating glomerular filtration rate (eGFR)>60 and 17 with eGFR<60 mL/min per 1.73 m2, uNGAL–reference interval <132 μg/L)2.The incidence of chronic kidney disease was determing using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations in eGFR. The study protocol complies with the Declaration of Helsinki, and was approved by the local ethics committees. Data are expressed as means ± SD for normally distributed values, as median with range for non-normally distributed values, and percentage using the Statistics for Windows program. To investigate the relation between urinary NGAL with renal function parameters data were also stratified in groups of eGFR and information of acute kidney injury (AKI) in the past. Statistical analysis was performed by statistical package STATA/IC ver.11.1. 60.6% of RHp have eGFR >60 ml/min/1.73m2, while a 39.4% of patients have eGFR <60 ml/min/1.73m2. Levels of uNGAL were 67,91 (6,4-415,5) ug/L in RHp with eGFR<60 and 85,69 (5.1-509.9) ug/L in RHp with eGFR≥60ml/ min per 1.73 m2. There were no significant differences between levels of uNGAL and eGFR values in patients with RH (p=0.192).

Levels of uNGAL and AKI in the past were 145.2 (36.1-501.9) ug/L in 8 RHp with eGFR<60 ml/min per 1.73 m2 and 197.4 (26.2 -509.9) ug/L in 3 RHp with eGFR≥60 mL/min per 1.73 m2 (uNGAL – reference interval <132 μg/L). Higher value of uNGAL in some RHp could have link in the repair stage after AKI and would reveal pathways that could link AKI and CKD in the future.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE ON URINE OMICS AND NEPHROMICS (URINOMICS 2017)

The Shrunken Pore Syndrome: Proteomics for further studies

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ABSTRACT

The recently described "Shrunken pore syndrome" (SPS) is characterized by a difference in glomerular filtration rate (GFR) between cystatin C and creatinine. The Shrunken pore syndrome, defined as an estimated $\mathrm{GFR}_{\mathrm{cystatin}}$ C less than 60% of their $\mathrm{eGFR}_{\mathrm{creatinine}}$, is found in about 8% of the adult Swedish population and is associated with higher mortality after coronary artery bypass grafting.

Our hypothesis is that differences in permeability for small and middle molecules indicate an early damage in the vessels of the kidneys and may be a common pathophysiological mechanism for damages in the heart and kidneys. We also investigated individuals hospitalized for the diagnosis of heart failure and found significant associations with measurements of right ventricular (RV) systolic function; (TAPSE and RV S´) (according to the equation pair $\mathrm{CKD\text{-}EPI}_{\mathrm{cystatin}}$ C and $\mathrm{CKD\text{-}EPI}_{\mathrm{creatinine}}$). We will use proteomic techniques with aptamers to study the whole human plasma proteome in relation to GFR estimated from iohexol clearance in 700 patients. GFR ranges from 8-119 mL/min/1.73 m2 and the aptamer technique has the capacity to determine 2900 proteins. Preliminary we can show differences in filtration for not only creatinine and cystatin C in these patients but also for beta trace protein. These studies will give new insight into the relation between levels of plasma proteins and GFR.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE III INTERNATIONAL CAPARICA CONFERENCE ON URINE OMICS AND NEPHROMICS (URINOMICS 2017)

Prostasin, the epithelial sodium channel activator, in urine of hypertensive patients and healthy subjects: relationship with aldosterone and ENaC function

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ABSTRACT

Prostasin is a serine endoprotease that is glycosylphosphatidylinositol (GPI)-anchored to the surface of epithelial cells and that is released in urine. This protease is involved in the epithelial Na channel activation [1] and a direct association between urinary prostasin concentration and the activation of the aldosterone-driven pathway has been suggested by *in vitro* and *in vivo* studies [2]. Studies in humans are few but they only could give clinical information about the relationship between prostasin, aldosterone and ENaC function. Studies on hypertensive patients are particularly informative, especially in the case of secondary forms characterised by excessive aldosterone excretion. Prostasin has, in fact, being suggested as a surrogate marker of aldosterone-dependent ENaC activity. We investigated u-prostasin concentrations in patients with primary aldosteronism, in patients with essential hypertension and in healthy subjects, to explore the correlation between prostasin and aldosterone. Methods: A total of 118 patients (62 primary aldosteronism, 56 essential hypertension patients) and 43 healthy subjects were enrolled. Biochemical and hormonal parameters were measured by applying routine laboratory methods, u-prostasin levels were assessed by ELISA and exosome prostasin levels by western immunoblotting. Results: Urinary prostasin was detectable and measurable in all samples. We could detect prostasin also in urinary exosomes. In healthy subjects urinary prostasin was similarly present in both genders, and it was not affected by the hormonal different phases of the menses. Prostasin was modulated by urinary Na, and prostasin levels appeared to be correlated with the aldosterone-to-renin ratio (ARR). Primary aldosteronism patients had higher u-prostasin levels than did essential hypertension patients. Prostasin levels were positively correlated with the ARR and inversely correlated with plasma K and urinary Na levels. Prostasin levels in the highest concentration quartile were associated with a several-fold higher probability of primary aldosteronism diagnosis in hypertensive patients,. Prostasin was specific but poorly sensitive as a diagnostic marker for primary aldosteronism by ROC curve analysis. Conclusions: Our data show that an elevated urinary prostasin concentration in humans is a specific marker for primary aldosteronism, confirming the involvement of the classical model of epithelial Na channel activation

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Acknowledgments: The work was performed in part at the LURM (Laboratorio Universitario di Ricerca Medica) Research Center, University of Verona. This study was supported by research grants from the Ministero dell'Istruzione dell'Universita` e della Ricerca (MIUR).

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SPECIAL ISSUE

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(FORENSICS 2017)

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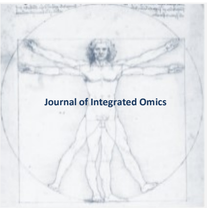

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Applying Penetration Test to Improve Cybersecurity Forensic Investigation

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Available Online: 15 December 2017

ABSTRACT

A data breach occurs when an unauthorized hacker accesses a compromised database or repository over a network connection. Cybersecurity forensic investigation seeks to explain how an Internet data breach occurred and who perpetrated the attack. There is an increasing need to develop techniques that could permit preliminary investigations for first responders at crime scene. This paper uses penetration test to model the investigative process, and engages digital evidence acquired from tester/target computers. All records are stored and collected from the following software [1]: Winfingerprint, Superscan, Nmap, SoftPerfect Network Scanner, Httrack, Nessus, The Analyzer Pro, and Wireshark. In Figure 1, Wireshark packets are filtered and retrieved in SQL database to compare with the auditing records in web servers. Their results are further analyzed to find some attack patterns in these digital records. It discusses how many available attack tools can be used to initiate a cyber intrusion. It also describes a prototype strategy in cybersecurity forensic investigation that is currently under development.

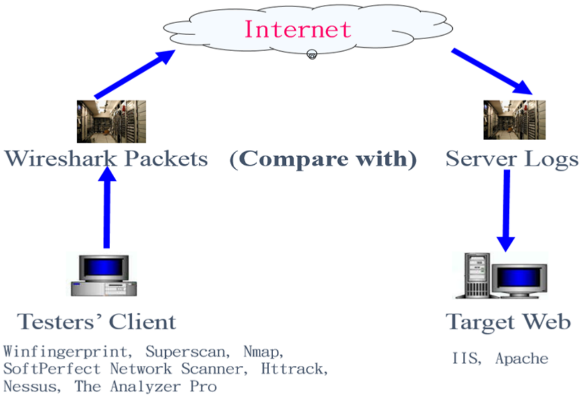

Figure 1. The architecture of penetration test platform

Keywords: Cyber Forensics, Digital Evidence, Wireshark Packets, Penetration Test

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Acknowledgments: This research was partially supported by the Ministry of Science and Technology of the Republic of China under the Grants MOST 106-2221-E-015-002-.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

A Network-Based Sniffer Architecture for Cybercrime Investigation

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ABSTRACT

Using sniffers becomes one of the most common approaches to examine the captured traffic activities when it comes to collecting digital evidence in cybercrime investigation. The storage and handling of sniffer packets are creating significant challenges for law enforcement agencies. These challenges include processing massive amounts of packets, maintaining the integrity of digital evidence, and storing digital evidence during the period of investigation [1]. The ISO/IEC 27043: 2015 international standards provide instructional guidance from the following processes [2]: readiness, initialization, acquisitive, and investigative. In Figure 1, this paper proposes a network-based sniffer architecture that helps address these issues and formalizes what should be logged for an appreciate cybercrime investigation. It also meets the need of ISO/IEC 27043:2015 standards to avoid fear, uncertainty, and doubt. The utility of this methodology is illustrated by applying it to a real-time network forensics and lawful interception system in Taiwan.

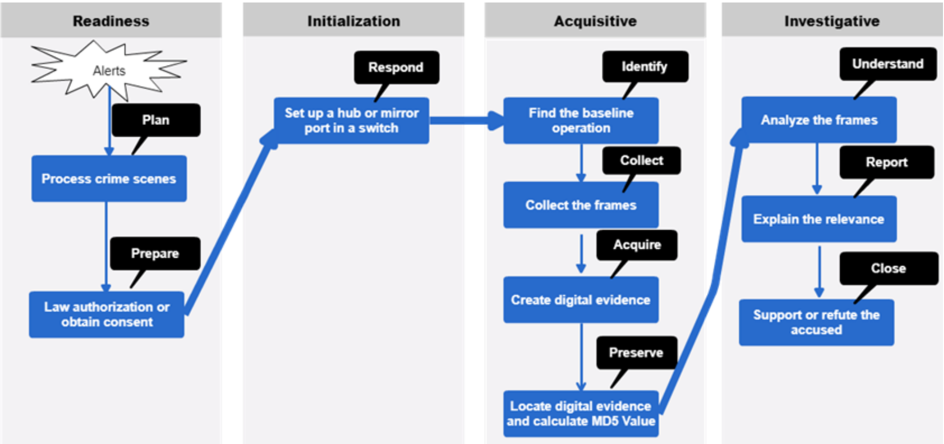

Figure 1. A network-based sniffer architecture for cybercrime investigation.

Keywords: ISO/IEC 27043: 2015, Cybercrime Investigation, Digital Forensics, Packet Analysis

Acknowledgments: This research was partially supported by the Ministry of Science and Technology of the Republic of China under the Grants MOST 106-2221-E-015-002-.

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JIOMICS | VOL 7 | ISSUE 2 | DECEMBER 2017 | 54–110

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

The Diplomatics and Digital Forensics Science in the digital records: The Pursuit of Authenticity

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ABSTRACT

The project aims at the application of Digital Diplomatic Science and Digital Forensics Science in digital born records [1], in order to guarantee its authenticity in institutional routines and processes, but also as a source of proof in possible legal and legal scenarios [2]. In view of the vast and fruitful discussions focused on the complexity of the digital records in Archival Science and Information Science I.C., there is a gap to manage and preserve the digital records, keeping them reliable, accurate and authentic in systems that have the same conditions. This gap makes it impossible to preserve records in the long term due, firstly, to the fragility of the systems where they are stored and, secondly, to the constant risks of obsolescence of hardware and software that occur on a day by day. Digital Diplomatic Science assists this process by determining the form and content of the record to evidence its legal-diplomatic authenticity and establish its historical value [3]. Digital Forensic Science, in turn, provides support for the chain of custody to remain intact, regardless of the medium on which the digital record is fixed. Is questioned: Is it possible to apply the practice of law and the Digital Forensics in the area of the archival science and information science, guaranteeing the authenticity of the born digital record? [4]. In addition, in what way does the junction between Digital Diplomatic Science and Digital Forensic Science guarantee the preservation and preservation of the born digital record? To answer such questions, this research proposes to link five areas of knowledge following the scientific experiences conducted at the University of British Columbia in Canada by the InterPares (International Research on Permanent Authentic Records) group [5]: Digital Forensics, Diplomatics, Archival Science, Information Science and Common Law. Areas of knowledge that are offering interesting results for the preservation not only of the record, but also of the social memory. The research will be carried out in a theoretical and qualitative way, taking into account the literature shared in the site Digital Records Forensics Project[6], coordinated by the author Luciana Duranti of the InterPares group, and the bibliography produced in the country of the areas of knowledge already referenced. The results obtained will contribute to the realization of alternative researches in document conservation within the Archival Science and the Information Science [7].

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Forensic DNA analysis of the biological objects sampled from the poaching sites in Belarus

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ABSTRACT

Motivation and Aim: The illegal removal of animals from the natural environment represents a world problem. Crimes against wildlife objects (in particular illegal hunting) have a high level of latency. DNA analysis of biological traces from the shooting or butchering site, clothes of the poacher, vehicle or meat storage sites may provide evidentiary information.

Methods and Algorithms: We performed multiplex PCR of STR loci using fluorescently labeled primers. PCR products were analyzed with capillary electrophoresis using automated sequencers manufactured by Applied Biosystems, PCR-RFLP.

Results: DNA analysis of wild animal biological traces is more complex than the DNA analysis of human biological traces because of the phylogenetic relationship of wild species to each other (for example, moos, roe deer, red deer) and kinship with domestic animals (bull, goat, sheep). Thus the obligatory stage of wild animal DNA genotyping is to solve additional classification problems of species affinity and differentiation between the wild or domestic animal. Based on the study of the phenomenon of cross-species amplification we developed a procedure for determining the animal species (moos, rode deer, red deer, deer) by genotyping of biological traces. The panels of loci for DNA identification of individual animal among these species have been chosen. Identification of individual specimen of moos and roe deer whose detailed genome structure is unknown was carried out on the basis of cross-species amplification. Polymorphism of 20 STR-loci and SNP-polymorphism of the melanocortin 1 receptor (MC1R) gene and nuclear receptor subfamily 6, group A, member 1 (NR6A1) gene [1] were studied in wild boar populations (719 samples) and herds of domestic pigs (304 samples, 6 breeds). On the basis of the results obtained a procedure for differentiation of the specimen origin from the wild boar or a domestic pig and procedure for individual identification of the specimen have been developed.

Conclusion: The study of genetic features of regional (local) populations of wild animals provides a means of successful identification of the objects from poaching sites. A number of expert examinations were performed with the samples of elk, deer, roe deer, wild boar, bison, bear, beaver, dog, hyena dog, fox, bull, horse, sheep, domestic pig.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Postmortem changes and Interpretation of Amphetamine type stimulants

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ABSTRACT

Amphetamine type stimulants (ATS) refer to a group of drugs that have a common phenethylamine structural backbone and include drugs such as amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA) and 3,4-Methylenedioxymethamphetamine (MDMA). The abuse of Amphetamine type stimulants (ATS) has emerged as a global problem. The abuse of these potent stimulants began to appear in a few countries of North America, Europe and the Far East, gradually spreading to neighboring countries in the respective regions as well as to other regions. By the mid-1990s, abuse of ATS has become a global phenomenon. Global demand for amphetamines, which increased strongly in most part of the world in the 1990's, is now showing signs of an overall stabilization, however its abuse is still prevalent all over the world while ecstasy use has been declining globally. There are distinct regional abuse patterns in ATS abuse. Methamphetamine is abused in north America and Asia, while amphetamine and Ecstasy in Europe and Australia. In Korea, methamphetamine is the most abused drug. Recently, along with the advances in organic chemistry that greatly enhance the capability of chemical synthesis, many synthetic drugs with psychoactive properties (New Psychoactive Substances, NPS) have emerged on the illicit market.In this talk, firstly the abuse trends of ATS and NPS with phenethylamine structural backbone will be discussed based on the report of UNODC. Secondly, the analysis of ATS and synthetic amphetamines in urine and hair will be focused because it has been a great challenge for forensic toxicologists due to insufficient chromatographic separation of their isomers and metabolites. Amphetamine and methamphetamine are chiral molecules and D-enantiomer has greater biological activity than the L-enantiomer. Lastly, postmortem changes and interpretation of ATS in drug testing will be discussed with real cases. There are various factors to have an impact on the concentration of ATS in postmortem specimens such as postmortem redistribution. The concentration of methamphetamine detected in cardiac blood was about 2 times higher than that detected in peripheral blood. Considering all these factors, the interpretation of toxicological results should be coupled with autopsy findings, crime scene information, related medical history and toxicokinetics.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

The determination of cholinesterase activity using photography

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Abstract

Smartphones are popular devices frequently equipped with a sensitive sensor like camera and immense computational ability [1,2]. Surprisingly, no serious application based on a smartphone in analytical chemistry is available except of remote control of another device. In this work, smartphone is performed as a tool for the determination of cholinesterasemia i.e. the determination of a biochemical marker butyrylcholinesterase (BChE). Paper strips soaked with indoxylacetate were used for the determination of BChE activity. Standard spectrophotometric test was used as a reference measurement. In the smartphone based assay, BChE converted indoxylacetate to indigo blue and coloration was photographed using integrated camera. RGB color model was analyzed and color values for the individual color channels were determined. The assay was verified using plasma samples and samples containing pure BChE, and it was validated to standard spectrophotometry. The smartphone assay was proved to be reliable and applicable for routine diagnoses where BChE serves as a marker. Liver function test and diagnosis of poisoning by some neurotoxic compounds can be exampled. It can be concluded that practical applicability of the assay is expected because of the results relevance.

Acknowledgments: This work was supported by the Ministry of Defence of the Czech Republic - long-term organization development plan Medical Aspects of Weapons of Mass Destruction of the Faculty of Military Health Sciences, University of Defence.

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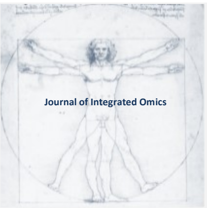

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Investigation of suicide risk factors in Yerevan, Armenia

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ABSTRACT

B**ackground:** According to the WHO almost one million people commit suicide around the world every year. Suicide remains a significant social, public health problem [1] and the number of suicides is increasing each year. The crucial role in suicide prevention is to understand the causes of suicide and draw attention to the risk factors. The purpose of the study was to investigate forensic medical and socio-medical aspects and methods of suicides, to specify potential risk factors, to develop and implement appropriate suicide prevention strategies by means of decreasing risk factors and increasing protective measures.

Materials and Methods: Data for this study were obtained from internal database of the Scientific Practical Center of Forensic Medicine, SNPO, Department of Forensic Medicine, City of Yerevan, for the period from 2003 to 2012. The study included 729 cases of suicide and was based on 10- year collected data screening. The relevant conclusions were made and suicidal risk factors were identified. Suicides have been investigated according to the following criteria: sex, age group, month, year, season and meteorological factors, place where the suicide was committed, alcohol or drug abuse, method of committed suicide, citizenship and nationality of suicide committed person, social status and social factors, mental disorders and somatic diseases.

Results: 1) Suicide is generally most common among those over the age of 70 (19%); however, those aged between 18 and 29 are at highest risk (18%); **2)** The risk of suicide is higher in spring (about 29.7% of all investigated cases), predominantly in May (about 10.8% of all investigated cases); **3)** The risk of suicide is higher in people with alcohol abuse (about 20.6% of all investigated cases); **4)** The risk of suicide is higher in people with mental disorders and somatic diseases (about 22% of all investigated cases); **5)** The risk of suicide is higher in people with many complex socio-cultural factors (about 19% of all investigated cases); **6)** The risk of suicide is higher among the unemployed (about 46% of all investigated cases).

The study showed that other factors affecting the risk of suicides, such as place of suicide, citizenship and nationality, drug abuse, were not significant and thus were not considered as suicidal risk factors.

Conclusions: The analyses of data collected for the period from 2003 to 2010 revealed the following major suicide risk factos—**1)** The highest suicide rates are reported for males at the age 70 and older, the persons between age 18 and 29 are at highest risk; **2)** Alcohol abuse; **3)** Spring months, especially May; **4)** Mental disorders and somatic diseases; **5)** Social factors and unemployment.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Use of Optical Coherence Tomography (OCT) on detection of postmortem Ocular findings: pilot data from two cases

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ABSTRACT

Purpose. The aim of this study was to describe by means of a portable Optical Coherence Tomography (OCT) the postmortem ocular findings in two cases of forensic interest.

Case 1. A 41-year-old Caucasian man, dead from a gunshot in the head, was found inside his car. Time of death was precisely assessed from the testimony of eye witnesses. The body was transported at the Morgue of Medico-Legal Institute at the University of Cagliari for autopsy. OCT scans of cornea, anterior chamber and retina were performed at the 6th, 12th and 24th hour postmortem without change eyelid opening status. Corneal examination showed a progressive tendency of tissue to thickening. From a morphological point of view, we also observed a progressive formation of waves in the posterior stroma and in the endothelium. An ongoing modification in reflectivity between anterior (hyper-reflective) and posterior (hypo-reflective) segment of the corneal stroma was detecetd and a progressive decrease in amplitude of anterior chamber, mainly related to changes in tissue curvature. The retinal tissue showed since the first scan an increasing trend of retinal oedema togheter with a diffuse vasal depletion. It was also detachable the formation of a scleral *tache noir*.

Case 2. A 42-year-old woman, dead from myocardial infarction, underwent an autopsy at the Medico-Legal Institute at University of Cagliari (as suspected case of medical malpractice). Exact time of death was deduced by certificate of death drawn up by the emergency medical team. Scans of cornea, retina and anterior chamber were executed at the 24th, 36th and 48th hour postmortem. From the first scan an enhancement of corneal thickness, if compared to the physiological *in vivo* ranges, was detected; a change in corneal curvature was observed while no images form the retinal tissue was achieved. Late scans evidenced a progressive corneal endothelial exfoliation, and an enhancement of posterior stroma waving.

Conclusion. Portable OCT may be an useful device to observe and to record postmortem ocular changes. Its use could represent an important tool to study the early and the late modification of ocular tissues, with a special interest in the forensic scenario (PMI estimation) and in ophthalmology (viability of cornea for transplantation purposes).

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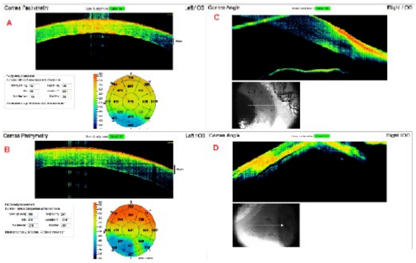

Figure 1. Pachymetric corneal changes over the time (A,B). Corneal angle (C) and OCT image of scleral *tache noir* (D).

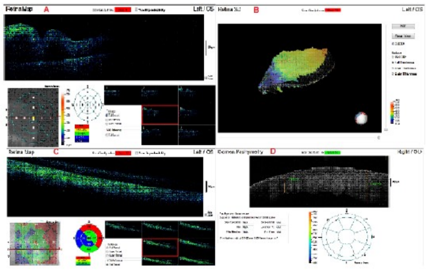

Figure 2. OCT image of the fovea (A). 3-D retinal reconstruction (B). Retinal map (C). Corneal tardive scan [>48h] (D).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

A rapid multi-target screening in urine for emergency toxicology by GC-MS and LC-MS/MS

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ABSTRACT

In order to establish the rapid method for screening of multiple toxicants in Emergency toxicology, GC-MS and LC-MS/MS were used to determine targeted and unknown toxicants in urine collected from intoxicated patients in emergency room. Totally 265 urine samples from February 2015 to March 2017 were submitted from Chungnam University Hospital emergency room. Urine samples were cleaned up by using Waters Ostro (pass-through type) and analyzed by Agilent GC-MS and LC-MS/MS. The library search for unknowns was conducted by in-house Mass spectral databases with the Automated Mass spectral Deconvolution and Identification System (AMDIS) as well as Chemstation software. For the specimen by LC-MS/MS, the 3200 Qtrap LC-MS/MS and Cliquid software (AB scx) was used for a simultaneous multi-targeted screening. As a result, a rapid multi-target method by GC-MS and LC-MS/MS was developed to determine the toxic substances in urine. By using Ostro extraction and in-house data base, it was possible to screen urines for toxic substances within three hours. Among 265 urine samples were examined, Zolpidem, acetaminophen and escitalopram were detected in 49, 29 and 16 cases respectively. Zolpidem was the most frequently encountered drugs in emergency room patients. By AMDIS & Chemstation with GC/MS and Cliquid 2.0 software with LC-MS/MS, unknown toxicants were well searched and identified. The comparison of urine analysis and the family report revealed that it is necessary to examine the specimen for the reliability of information. The rapid multi-target screening methods by GC-MS and LC-MS/MS proved to be well applicable to the hospital poisoning samples. This method will efficiently use to detect toxic substances in urine within 3 hour in emergency cases.

Acknowledgments: This work was supported by a grant (MFDS 2016-22204) from Ministry of Food and Drug Safety in 2017.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

A molecular population genetics approach for the identification and forensic assignment of viral transmission groups

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ABSTRACT

Most cases of virus transmission that get to court imply pairs of putative donor-recipient. They usually involve fast evolving viruses, such as Human Immunodeficiency Virus (HIV) or Hepatitis C Virus (HCV), capable of accumulating enough variation within an infected individual to generate a heterogeneous swarm of virus variants usually known as "viral quasispecies". The viral population in the recipient is usually a subsample of that in the donor individual and, if the time elapsed between the infection event and the sampling for analysis is not long, they can be easily identified as such. However, there can be a long delay in the diagnostic of infection for both viruses, which allows for intrapatient evolution and divergence of the two populations. With longer times between infection and sampling, the donor's and the recipient's viral populations may reach complete differentiation and their relatedness can be inferred only through phylogenetic analysis including some unrelated, control samples. A high bootstrap support is usually accepted as a reliable indicator of a close link, resulting from a transmission event. However, this depends on the availability of appropriate controls and the situation can worsen when the transmission under investigation is not between a pair of individuals but results from many such events. We analyzed one such case [1] which involved the infection of almost 300 persons from a common source, a practicing anesthesiologist. There, we included the analysis of within patient variability to better determine the estimated time of infection as well as to provide individualized estimates of the probability of each person having been infected by the source. This analysis included local controls (n=42) and putative outbreak victims (n=47) which were effectively excluded from the outbreak. The increased availability of next generation sequencing allows a more efficient and affordable estimation of within patient variability which we have recently used to establish which persons infected by HCV were likely members of transmission clusters despite the initial results with the analysis of a portion of the NS5B gene with a local population controls [2]. We present a population genetic parameter that can be estimated from intrapatient data (obtained by sequencing of clones or PCR products or by NGS) and used to discriminate between epidemiologically relevant clusters of transmission at different times in the past, through comparison with data from unrelated controls. This parameter is very flexible and, in the absence of additional epidemiological information and with additional phylogenetic analyses, can be used as a first guide for establishing hypotheses of which patients can be included in a transmission cluster.

Acknowledgments: Supported by projects BFU2014-58565R (MINECO, Spanish Government) and PROMETEO/2016/122 (Generalitat Valenciana, Spain).

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

DSC as a new diagnostic method in forensic medicine

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ABSTRACT

Differential scanning calorimetry (DSC) is an often used method in thermal analysis. Recently, growing clinical use for it. By this method we can measure the effects of drugs/medicines and we can characterize changes in body. We have examined the effects of cyclophosphamide on different samples of guinea pigs to give a judicial expertise in a disability pension lawsuit. Besides of its beneficial effects cyclophosphamide may have got severe life-threatening side effects and complications because of the actual plasma level and high cumulative dosage. In the first step of our experiment we examined the effects of cyclophosphamide on guinea pigs' nerve-muscle complex with oncological indication by using a dosage protocol based on body mass [1]. According to our results we could show a significant, dose dependent difference between thermal parameters of untreated and treated samples which proved that cyclophosphamide has got a nerve and muscle damage effect. As a second step with the same method and drug dosage we examined its effect on the heart muscle [2]. The third step was in the further clinical application experiment with unchanged parameters on blood plasma as well as blood cells too, exhibiting dosage dependent changes on plasma and blood cells [3]. Evaluating the per-formed studies a correlation with the dosage can be observed on different experimental materials. We found detectable alterations with DSC on blood plasma components too, so it can be used in clinical routine. In long term treatments incidental severe results and side effects caused by cumulative dose may become predictive with this method. If we can manage to predict the harmful effects for patients which are arising from different factors, we could prevent them with this method by decreasing the dose or changing to other drug. All these show a new promising area in DSC usage which passed out of mind in the last 10 years.

Keywords: cyclophosphamide, nerve, muscle, blood plasma and cell, DSC

Acknowledgments: This presentation is dedicated to the 650th anniversary of the foundation of the University of Pécs, Hungary.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Forensic EcoGenomics – The successful application of microbial ecology techniques for enhanced forensic analysis

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ABSTRACT

Developed in 2014, the term 'Forensic EcoGenomics' was defined in literature as "*the application of molecular microbial ecology techniques at the interface of (environmental) forensics, microbiology and archaeology*" [1]. It is inherently trans-disciplinary by definition and application, and is one of several emergent forensic sub-disciplines that aim is to advance forensic science and enhance the modern crime scene toolkit. Therefore, forensic ecogenomics encapsulates culture-based and molecular techniques that analyse the dynamics of microbial and macrobial communities in different forensic contexts. Although we have proposed that forensic ecogenomics has a significant role in ante- and postmortem investigations, this paper will focus on our subsurface decomposition-based research [e.g. 2]. Since most studies have to date focused on aboveground scenarios, this discourse will explore shifts in the soil necrobiome particularly belowground due to the decomposition of *Sus scrofa domesticus* – a widely accepted surrogate for human cadaver. Further to this will be comparisons of soil bacterial and fungal community structure and composition during the decomposition of different types of plant litter. The aims of the overarching research programme have been to apply denaturing gradient gel electrophoresis and next generation sequencing to: (i) measure shifts in soil necrobiome community composition and structure in the presence of a mammalian analogue; (ii) compare these to plant litter decomposition; and (iii) assess the potential applicability of forensic ecogenomics-based analyses in pilot studies and, ultimately, real crime scenes

Acknowledgments: The Teesside University Research Fund is acknowledged for funding Project 10/06/07. Professor Tim Thompson, Dr Helen Carney, Dr Gillian Taylor and Dr Caroline Orr are acknowledged gratefully for their co-supervision of Messieurs Olakanye and Bisker.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Deadly head injuries by military weapon: a special attention

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ABSTRACT

Gunshot wounds have a very special appearance because of their penetrating nature. They have a great diversity of lesion depending on the weapon, the ammunition, the distance and the angle of the shot, as well as the living target.

On the human head, these injuries are characteristic of specific lesions related to the structure of the cranial case, it's require special attention during the external examination and autopsy to help determine the medico-legal form of the wound.

We Report in this work, our experience in head injuries by assault rifle, since 2011 at the autopsy laboratory of the Military Hospital of Tunis, Tunisia, and the difficulties encountered in examining these wounds.

All the head injuries observed are caused by the Steyr Aug assault rifle, the main weapon used by the armed forces in Tunisia, and by the AK47 the weapon used during the terrorist attacks. Accidents and homicides are the majority forms. A suicidal case was reported but quickly converted into homicide after the autopsy data and the deep investigation.

The aim of our study is to emphasis the fact that autopsy remains an essential tool to reduce misdiagnoses in addition to assessing the gunshot characteristics.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

A Study on Physical Features and Potential Wounding Effects of Fireworks Which Is One Of The Samples of Pyrotechnics

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ABSTRACT

Aerial shells which is one of the pyrotechnic products are widely used in some religious and national days all around the world. Depending on the common use there are some scientific research and case presentations about wounding and deathes caused by aerial shells and other fireworks in literature. Use of aerial shells in Turkiye is less than other countries but becoming very common in recent years in public. In some public incidents and protests, it has been observed that aerial shells are used like a firearm against law enforcement. In this perspective, potential risks of aerial shells are researched this study. Commercially available aerial shells were physically examined for their construction and functioning mechanism, their extradiction from the barrels was recorded and speed calculated by fastcam, detonation of shells were recorded with fastcam and detonation pressure was measured, finally shells were detonated 19 cm in front of sheep eyes mounted on ballistic simulants and physical effects were inspected on simulants and pathological researchs were held on the sheep eyes. In 25 (%55,5) sheep eyes of 45 eyes used in experiments; foreign material, focal bleeding, extraocular muscle bleeding, bleeding, focal detachment, decolman and congession are observed in pathological analyse of the sheep eyes. Measured detontion pressure value of shells 89,64 kPA (SD: 19,241) and the fragmentataion effect whic is observed in fastcam records were found relative to traumatical observations on sheep eyes.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Recognition of the ‘high quality forgeries’ among the medicines: application of NIR spectroscopy and chemometrics

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ABSTRACT

Counterfeiting causes a huge economic and reputational damage to pharmaceutical companies, as well as poses a significant danger to public health. Fake medicines could be of different type: placebo, the medicines with lower concentration of active substances, the drugs that do not contain the proper concentrations or contain a wrong type of excipients, etc. From the recognition point of view there are also various types of fakes. They are (1) pills/tablets that can be recognized without any instruments, simply by glance, or, at least, by experienced glance; (2) medications with special drug packages, holograms, unique printing on tablet surface, special shapes of pills and capsules; (3) fakes that only can be detected using chemical/physical testing of drugs themselves. The most difficult for revealing are 'the high quality fakes’, which have a proper composition but produced by the underground manufactures with violation of technological regulations. For rapid testing we propose application of Near Infrared (NIR) measurements accompanied with chemometric data processing. NIR spectra carry information regarding not only chemical but also physical phenomena. A general approach is to consider a remedy as a whole object, taking into account a complex composition of active ingredients, excipients, as well as manufacturing conditions, such as degree of drying, etc. A newly developed classification method, DD-SIMCA, shows satisfactory results both in revealing counterfeits and in separation of various manufacturers of similar drugs. A real world example presents an analysis of the widely used medication for treating allergies, produced by five various manufacturers, and comparison the results with counterfeited samples (Figure 1). The case study demonstrates that theoretically predicted classifier characteristics, such as the Type I error, α, and the Type II error, β, are confirmed by the real-life calculations. The values of α and β errors provide a quantitative assessment of the risk of wrong decisions and can be employed for the science-based risk assessment.

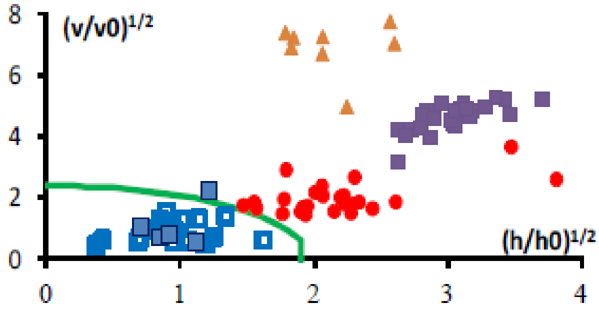

Figure 1. The DD-SIMCA plot. The green curve delineates the acceptance area. Outside objects are aliens.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE 1ST INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Quality Improvement for Criminal Investigations Lessons from Science?

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ABSTRACT

Criminal investigations generally aim at discovering previously unknown facts. The same is true for scientific (or academic) research. Both follow a rather tight framework of rules – most importantly, the principles of objectivity, reliability and validity. However, some of the intentions differ. Science generally attempts to discover and/or explain new principles, while criminal inquiries are instead usually bound to past, often singular, events. For example, the methods used in forensic investigations are required to be well established, standardised and undisputed inasmuch as possible. In contrast, the exploration of new methods is an important feature of the advancement of science. Consequently, both tendencies – similarities and opposites – can be discerned when comparing criminal and academic examinations.

The 'Pareto principle' indicates that the vast majority of all criminal investigations runs rather un-problematically. Nevertheless, the highest quality criteria must be guaranteed for these and the remaining, more challenging cases as well – based on the 'fair trial' principle. Acknowledging that mistakes are inevitable (Murphy's law), methodical approaches for error identification, handling, management and reduction are essential.

Error correction mechanisms that are typical for forensic statements normally include a second source of expertise and/or an appeals procedure. In academic science, however, the peer review system has long been established as the most important quality control and error correction system. In addition, possible mistakes can usually be corrected in later, more detailed studies. However, the central position of forensic experts and criminal investigators in a legal procedure and the severe personal consequences of incorrect statements emphasize the high importance of continuous improvement of both the qualifications of the investigators and the quality of their methods.

Nevertheless, error reduction provisions should not be restricted to technical measures like quality management and accreditations. Additionally, a systemic/organisational approach towards error management seems promising. This involves, among other measures, a systematic examination of mistakes and the recognition of the human factors that underlie them. Nevertheless, an indispensable component for quality enhancement is intense cooperation from both sides – the criminalistic and forensic practice as well as scientific (basic) research.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Rectus abdominis rupture - a case of occupational injury

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ABSTRACT

Because of its anatomical location and its verticality, the rectus abdominis is subjected to dynamic stretching and shortening movements. As such, it is the most prone abdominal muscle to injury. Despite this, lesions on the abdominal wall are relatively rare, being more frequent in sportsmen [1]. These lesions are vastly underdiagnosed and untreated because of the very scarce and insidious symptoms and the low functional limitation that they generate, leading to chronicity [2].

Small lesions are most frequently located in the bottom half of the rectus abdominis. The risk associated with these lesions is greater in low muscle mass and absent of pre-competition heating. The main injury mechanism are sudden or repeated trunk flection, extension and rotation or direct abdominal injury. Repeated Valsalva maneuver (intense cough, sneeze, vomit, effort to defecate) can also cause this injury [3].

The authors present a case of a 37-year-old man, bricklayer, who suffered a work accident when lifting a concrete beam experiencing severe pain in the lower abdomen. Two days later he went to a local hospital where initially he was thought to have a urinary tract infection. That diagnosis was ruled out by a urinary test, receiving discharge with probable rectus abdominis rupture. He started follow up in the clinical services of insurance company where an ultrasound confirmed a rectus abdominis rupture. Conservative treatment with rest was chosen.

Physical examination revealed a small deformity in the left inferior quadrant of abdomen, slightly painful to deep palpation, without herniation with Valsalva maneuver. Regarding the bodily harm parameters, a temporary total disability of 46 days was proposed. Taking into account the physical effort necessary for his normal work activity and the deformity observed upon physical examination a partial disability of 2% was assigned.

Despite the lack of reference to sequelae of rectus abdominis rupture treated conservatively in the *Tabela Nacional de Incapacidades para Acidentes de Trabalho e Doenças Profissionais* (national disability table), a percentage of disability was yet proposed, given that, it is the responsibility of the medical experts to depart from the regulation table whenever it seems appropriate, as long as duly justified, as was the case.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Acid attack in domestic violence – a case of serious physical harm

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ABSTRACT

Domestic violence against women is a global issue that transcends national, cultural, racial and class boundaries [1]. Many authors consider the research made on cases of abused children in the 60s the stepping stone regarding a model of domestic violence which included the child, but also the woman and later the elderly [2]. Incidents of domestic violence include battery, beatings, acid baths, rape, and even death through honor killings [3]. Acid thrown on the victim's face is a common form of assault in many countries. The purpose of these kind of assaults is not to bring death but to cause severe facial disfigurement or blindness. Moreover acid is often readily available and at an inexpensive cost. Regardless of gender or motive, the attacks fulfill their intended consequences, and the resulting scarring and deformities lead to disability, destitution, and social isolation [4].

The authors present a case of a 57-year-old woman, house cleaner, divorced after years of domestic violence inflicted by her ex-husband. She was attacked with acid. Of the assault resulted burning of the face, neck, shoulders and thorax. Primary care was undertaken by paramedics who washed the wounds and transferred the victim to a central hospital with a specialized burn treatment center (Coimbra). During hospitalization, the victim required band aid treatment every other day, sedation for balneotherapy and surgery (skin autograft from the anterior thigh to the neck and anterior thorax). The victim had a previous history of domestic violence with physical assault and threats with a firearm and acid, feeling terrified of her ex-husband. Physical examination revealed three scars on the face, a large area of scaring tissue involving the neck (retractile, limiting mobility) extending to the abdomen and a grafting harvest zone on the anterior thigh. According to Portuguese Penal Law this episode represents a serious physical harm as the lesions cause serious and permanent disfiguration, affect the ability to use one's body, namely cervical mobility, and cause a particularly painful disease. Medical-psychiatric follow-up and the adoption of psychosocial measures were recommended to ensure the victims treatment and protection.

In conclusion, it is important for the forensic doctor be prepared to analyze the problematic issue of domestic violence from a medical but also legal scope, making sure never to forget their social role to signal and facilitate the handling of cases as complex as domestic violence.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

An Automated Magnetic Dispersive Solid-phase Extraction Method for Detection of Cocaine in Human Urine

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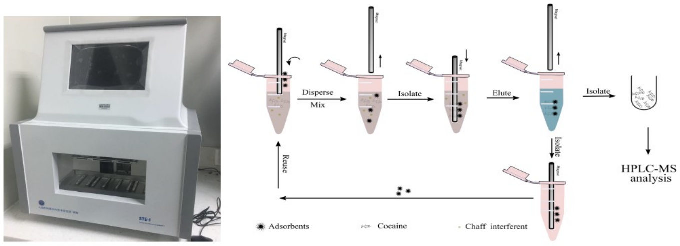
ABSTRACT

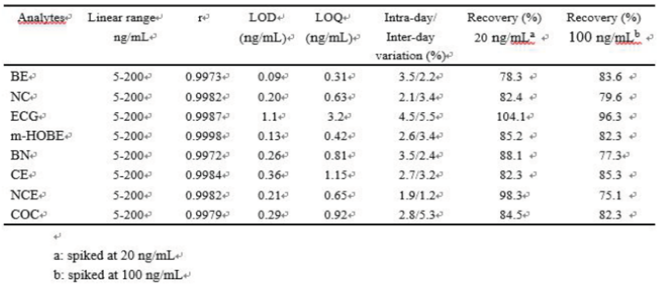
Growing consumption trend of abused cocaine and drug crimes are a great concern, therefore urine sample testing has become an important noninvasive sampling whereas cocaine and its metabolites (COCs) are usually present in high concentrations and relatively long detection windows. However, direct analysis of urine samples is not feasible and the extraction step is time-consuming. So developing a sensitive, rapid and high-throughput method for detection of COCs in human body is indispensable for law enforcement officers, treatment specialists and health officials. In this work, a new automated magnetic dispersive solid-phase extraction (MDSPE) sampling method followed by high performance liquid chromatography-mass spectrometry (HPLCMS) was developed for quantitative enrichment of COCs from human urine, using modified magnetic nanoparticles as absorbents. The proposed device significantly improved the sampling preparation efficiency with 32 samples in one batch within 40mins. Optimization of the preparation procedure for the magnetic nanoparticles was explored and the performances of magnetic nanoparticles were characterized by scanning electron microscopy, vibrating sample magnetometer and infrared spectra measurements. Several analytical experimental parameters were studied, including amount of particles, adsorption time, elution solvent, extraction and desorption kinetics, and the verification of the proposed method was accomplished. The limits of detection for the cocaine and cocaine metabolites were 0.09-1.1 ng·mL-1 with recoveries ranging from 75.1 to 104.1%. Compared to traditional sampling method, this method is time-saving and environmentally friendly. It was confirmed that the proposed automated method was a kind of highly effective way for the trace cocaine and cocaine metabolites analyses in human urine.



(a) (b)

Figure 1. Automated sampling equipment (a) and process of magnetic dispersive solid-phase extraction (b).

Table 1. Linear ranges, correlation coefficients ®, LOD), LOQ, intra-days/inter-days variation, recovery and RSD for COCs studied.



Analytes	Linear range ng/mL	r	LOD (ng/mL)	LOQ (ng/mL)	Intra-day/ Inter-day variation (%)	Recovery (%) 20 ng/mL^a	Recovery (%) 100 ng/mL^b
BE	5-200	0.9973	0.09	0.31	3.5/2.2	78.3	83.6
NC	5-200	0.9982	0.20	0.63	2.1/3.4	82.4	79.6
ECG	5-200	0.9987	1.1	3.2	4.5/5.5	104.1	96.3
m-HOBE	5-200	0.9998	0.13	0.42	2.6/3.4	85.2	82.3
BN	5-200	0.9972	0.26	0.81	3.5/2.4	88.1	77.3
CE	5-200	0.9984	0.36	1.15	2.7/3.2	82.3	85.3
NCE	5-200	0.9982	0.21	0.65	1.9/1.2	98.3	75.1
COC	5-200	0.9979	0.29	0.92	2.8/5.3	84.5	82.3

a: spiked at 20 ng/mL
b: spiked at 100 ng/mL

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International cooperation to counter cybercrime

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ABSTRACT

The topic of Cybercrime in international law branchs out in "cybercrime against individuals" and "cybercrime against states". The latter have not yet been disciplined by international law even if the number of cyberattacks against critical infrastructure is rapidly increasing, so nowadays targeted staes are still forced into the so called "response crisis" - which often develops in a real state paralysis - arising from the doubt that in case of a cyber threat or a cyber attack any operative choice may lead to a violation of international law and, above all, of the absolute prohibition of resorting to the use of armed force [1].

On the contrary some international legal instruments exist in the field of cyber crimes which are aimed at contrasting and persecuting private cyber offences: almost 125 countries have already signed and/or ratified a legal instrument on cybercrime (i.e. *Councile of Europe Convention on Cybercrime* (2001); *Shanghai Convention on Combatting Terrorism, Separatism and Extremism* (2001); *League of Arab States Convention on Combatting Information Technology Offences* (2010); *African Union Convention on Cybersecurity and Personal Data Protection* (2014)) [2]. This outcome is not positive mainly because there are too many instruments giving a partial regulation on the issue of cybercrime and, inter alia, with a scarce number of ratifications with the result that the international legal framework appears fragmented. Certainly the Council of Europe Convention on Cybercrime ("CEC") adopted in 2001is the milestone on the field: it is a law-enforcement treaty concluded with the purpose of harmonizing the domestic legislations of the contracting parties in the field of cybercrime and to establish effective transnational cooperation in contrasting, preventing and sanctioning said phenomenon. The CEC was considered as a supplemental instrument to the 1957 *European Convention of Extradition* and the 1950 *European Convention of Mutual Assistance in Criminal Matters* and the related *Additional Protocol* finalized to promote and simplify extradition procedures and to overcome many of the impediments of an internal nature (for example, the need for double incrimination as a conditio sine qua non for the purpose of extradition requests) which, as past experience has demonstrated, have allowed numerous criminals to escape justice (between 1982 and 2003, the much debated "*Mitterrand doctrine*" prevented the extradition from France to Italy of Italian criminals convicted of terrorism as the French President was against the anti-terrorist laws passed in Italy during the 1970s and 1980s). Nonetheless, considering that nowadays only 56 States ratified the Convention, despite the fact that the initial aim was that of creating a universal instrument, this treaty is deemed as a big failure, especially because international cooperation in fighting cybercrime has developed - and still develops - outside of the Convention. The reduced number of Signatories can be primarily attributed to the limit that concern every universal treaty, that is the difficulty involved in identifying contents to be agreed upon by all the States in the International Community [3]. Secondly the fact that a similar convention was introduced by the Council of Europe and not by the United Nations seems to constitute one of the main causes for reticence to accession, despite the fact that, formally, the Treaty is open to ratification by any States wishing to do so. [4]. This is the reason why "*national governments often cooperate with each other informally by exchanging information, investigating attacks or crimes, preventing or stopping harmful conducts, providing evidence and even arranging for the rendition of the requesting state*" [5].

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The Forensic application of proteomics for the study of the time of death: An operative experimental model for post mortem interval estimation

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ABSTRACT

‘Proteomics is a branch of molecular biology that allows the systematic identification of the proteome from a quantitative and qualitative point of view. Below, we propose the operating model of an experimental study currently underway at the Department of Legal Medicine of the University of Catanzaro. The model is based on taking of peripheral blood samples on patients who died at the Intensive Care Unit, following an operative protocol. The study was approved by the Ethics Committee of University. The informed consent was signed by the family members before the death of the patients. Samples were taken according to predefined time intervals, starting from the exact time of death (“time zero”) and up to two hours after the death. Samples were immediately centrifuged in order to extract plasma, stored at -80 ° C and they are currently subjected to proteomic analysis by Western Blot and Mass Spectrometry at Proteomic Laboratories of the University of Catanzaro. Although the experimental study is still ongoing, we expect to find consistent results both with the time interval examined and the data already known in the literature. In fact, a review of literature on this topic has already shown that several proteins can undergo quantitative changes in terms of increase or reduction directly proportional to the postmortem interval investigated, but also qualitative ones. According to the scientific evidence already available in the literature, the expected results of the study are related to the search for quantitative and/or qualitative alterations from the exact moment of death of some markers, already showing time dependent variations such as:

1. ubiquitous cellular proteins, like HMGB1 (High Mobility Group Box 1): this protein has already proved to progressively increase with respect to time [1];
2. specific organ proteins:
    - muscle proteins due to progressive degradation, such as cTn I and cTnT [2] [3];
    - proteins related to the brain damage, such as GFAP (Glial fibrillary acidic protein) or talin, respectively with an increase and a reduction.

Finally, this operating model is intended to:

- Identify the possible role in the estimation of PMIs of new potential protein biomarkers expressed in peripheral blood from the exact moment of death;
- Verify and evaluate in detail the variation of the proteomic profile of markers already known in the literature;
- Focus on the analysis of the so-called "early post-mortem interval" for forensic purposes

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Recognition of patterns in pattern recognition

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ABSTRACT

Classification, aka 'pattern recognition', takes an essential part in scientific research, including , a fortiori, the forensic applications. Classification method should be selected with respect to the underlying features of the solved problem. In practice, each sample that has to be attributed to a predefined class, or classes, or left unclassified. In case the sample is unlabeled, this is either identification, or discrimination problem. Otherwise, this is an authentication task. Another characteristic refers to the classes, which list can be exhaustive (complete), or open (incomplete). Our message is that a risk-based decision should only be made out after a thoughtful examination and recognition of the classification pattern (prototype: identification, discrimination, or authentication), which goes better with the research objectives. This time we focus on the assessment of the authentication (labeled, incomplete) versus the discrimination (unlabeled, complete) classification problems [1].

Authentication is the process of determining whether an object is, in fact, what it is declared to be. Discrimination is the process of allocation of an object to one of the predefined classes. In practice, authentication is often solved using discrimination. We explain that such techniques do a poor authentication job. The main drawback of these methods is inability of proper classification of new samples, which do not belong to any of the predefined classes. We illustrate this by real-world examples and a comparison of the two methods: Partial Least Squares- Discriminant Analysis, PLS-DA, and Data Driven Soft Independent Modeling of Class Analogy, DD-SIMCA [2, 3].

Pattern recognition encloses a big variety of different methods and techniques. Each type of problem requires an application of relevant methods. A well constructed discrimination method will perfectly classify a new sample only if this sample is a member of one of the predefined classes. However, in case the new sample does not belong to any of such classes, the discriminant analysis is unable to properly define the membership of the sample. Thus, discrimination methods are inappropriate for solving authentication problems. Class-modeling methods [4] develop the acceptance area around the target class, and, thus, delimit the target objects from any other objects and classes. This is the reason why only one-class classifiers should be used for authentication.

Acknowledgements: We acknowledge partly funding from the IAEA in the frame of projects D5240 and G42007.

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Recent advances in the analytical chemistry of cadaveric decomposition

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Available Online: 15 December 2017

ABSTRACT

The chemical processes of human cadaver decomposition are complex and not well understood. The study of decomposition chemistry aims to elucidate the postmortem processes, particularly relating to the production of volatile organic compounds (VOCs) throughout the various decomposition stages [1]. The study of human remains (HR) decomposition chemistry is further motivated by the need for developing specific tools to locate dead or injured bodies during mass disaster victim recovery cases, for forensic investigations, and the search for clandestine graves. Over the last few years, the use of thermal desorption coupled with comprehensive two-dimensional gas chromatography time-of-flight mass spectrometry (TD-GC×GC-TOFMS) has allowed partial elucidation of cadaveric VOC profiles [2]. GC×GC-TOFMS is a powerful Separation Science tool that combines advantages from the added peak capacity and zone compression of GC×GC and from the deconvolution of mass spectral signals of high resolution TOFMS offering accurate mass measurements for proper analyte identity assignments. The collection of exhaustive data sets including first and second retention time values (^{1}tR and ^{2}tR), intensities, mass spectra, accurate mass values, however, results in the production of large files that require specific treatment before they can be exploited [3]. On the top of basic spectral alignment and normalization, supervised and non-supervised statistics have to be used to extract the relevant information from the multidimensional perspective [4].

This lecture will illustrate how such data can be produced and used in specific forensic chemistry situations such as training of HR detection (HRD) canines [5], search for dead bodies [6], cadaveric internal gas reservoir analyses for postmortem interval (PMI) determination [7].

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Forensic engineering of advanced polymeric materials

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ABSTRACT

Classical forensic polymer engineering concerns a study of failure in polymer product. This area of science comprises fracture of plastic products, or any other reason why such a product fails in service or fails to meet its specification. Our novel approach i.e.: forensic engineering of advanced polymeric materials (FEAPM) deals with the evaluation and understanding of the relationships between their structure, properties and behavior before, during and after practical applications. FEAPM provides a central driving force for the otherwise disconnected works and should help to design novel polymeric materials and to avoid potential failures of the commercial products manufactured from them [1-4].

The selected prediction and case studies related with design of novel polymeric materials for diverse applications in medicine, cosmetic industry and agrichemistry will be presented. The molecular level characterization of polymers, including natural polyhydroxyalkanoates (PHA) and their synthetic analogues, will be discussed [5]. It will be demonstrated, that both the ex-ante investigations as well as the ex-post studies are needed in the area of FEAPM in order to increase efficiency and to define and minimize the potential failure of novel polymer products before and after specific applications.

Acknowledgments: This research was partially supported by the National Centre for Research and Development in Poland within project APOLLO 2/269760/1 NCBR/2015 (STRATEGMED) and PELARGODONT Project financed under the M-ERA.NET 2 Programme

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Molecular biology aspects of hypothermia

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ABSTRACT

Cold exposure causes a multitude of molecular responses in cells, tissues and systemic level. Some of the stress reactions connected to hypothermia are beneficial, protecting tissues against cold-induced acute ischemia. Cardiac cell survival has been extensively studied in animal models and cell culture, but limited information is available from hypothermic humans. We have recently successfully identified molecular events connected to hypothermia, cardiovascular diseases and other deaths by investigating human postmortem tissue samples, especially the heart with various methods. We have also carried out precise cold exposures of rats to analyze effects on target genes during different levels of hypothermia. Protein as well as RNA level studies have been carried out using quantitative PCR, immunohistochemistry and ELISA-assays, for example. Activation of sympathoadrenal axis is characteristic to hypothermia and leads to high levels of adrenalin and noradrenalin in the circulation. These catecholamines modify e.g. endothelial function with gene expression changes dependent on the severity of cold exposure [1]. Typically, gene expression profiles vary in tissue-specific manner during hypothermia [2]. We have also identified stress-specific cardiac expression pattern of certain transcript variants in hypothermia, chronic hypoxia and acute ischemia [3]. Cell-cycle regulator p21 cyclin-dependent kinase inhibitor, endothelial thrombomodulin and growth factor amphiregulin are examples of target genes and proteins in our studies. We have investigated the values of adrenaline to noradrenaline ratio [4] and thrombomodulin [5] as markers of antemortem hypothermia. Tissue samples from medico-legal autopsies and animal experiments together with cell culture studies help us understand the molecular basis of hypothermia further, aiming to improve postmortem diagnostics.

Acknowledgments: The authors are grateful to the forensic pathologists, autopsy assistants and laboratory technicians for collaboration.

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Non-Destructive Identification of Defaced Serial Numbers on Metal Surfaces

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ABSTRACT

Infrared thermography is useful for nondestructive evaluation of structural integrity. Subsurface defects produce non-uniform heat dissipation and this phenomenon is captured by an infrared camera. Pulsed thermography has emerged as the most widely used technique where the specimen surface is heated with a brief pulse of heat and surface temperatures are monitored with an IR camera. In this study, lock-in thermography (LIT) is used to recover defaced serial numbers based on the underlying deformation from the stamping or laser etching process. For example, the collection of LIT phase images in Figure 1 are unfolded and decomposed by principal component analysis (PCA) to form a sequence of principal component score images shown in Figure 1 for a defaced number 6. In order to avoid selection of specific score images for further examination, all images are used in a data fusion approach for a consensus analysis. In order to identify a defaced number, multiple numerical library images are matched to each score image by a collection of similarity measures. Prior to computing similarity measures between a library number image and a defaced score image, all images are decomposed to Zernike moments by respective Zernike polynomials. Zernike polynomials form an orthogonal basis set allowing extraction of image features describing shape characteristics of an imaged object. Fusion is again the main tool for analysis of the multiple libraries and similarity measures providing consensus identification of a defaced number. Results are presented for a series of known defaced numbers on stainless steel as well as recovery of the VIN defaced on a stolen motorcycle.

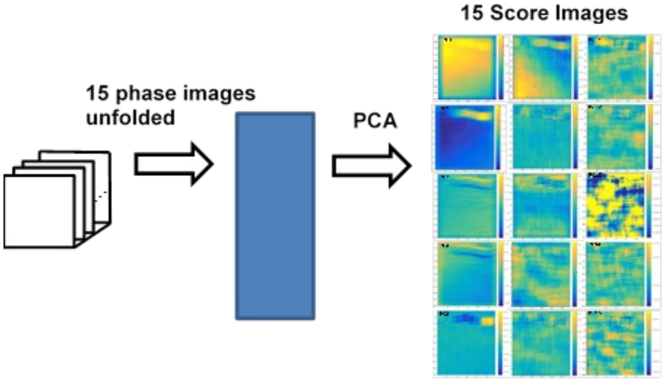

Figure 1. Phase images unfolded for PCA to form 15 score images.

Acknowledgments: This material is based upon work supported by the National Institute of Justice Grant NIJ 2013-R2-CX-K012 and is gratefully acknowledged by the authors .

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Tissue glycogen and beta-hydroxybutyric acid in lethal hypothermia

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ABSTRACT

Biochemical studies in hypothermia are widely used in forensic medicine. In our country glycogen content in the skeletal muscle, liver and heart muscle of all suspected hypothermia cases is determined by spectrophotometric method. But it is known that the cases of death from hypothermia are usually accompanied by increasing of beta-hydroxybutyric acid concentration. The purpose of our study was to compare the significance of the determination of glycogen content and beta-hydroxybutyrate in lethal hypothermia. The sample of blood and the fragments of liver, skeletal muscle and heart muscle were collected during autopsies from 13 men and 5 women aged 21-81 who died in the circumstances suggesting overcooling. In 11 cases (alcohol content in the blood was 1,1-2,4‰) the glycogen level in the liver, skeletal muscle and heart muscle was reduced, the beta-hydroxybutyrate concentration was increased and there were microscopic signs of hypothermia. In 1 case (alcohol content in the blood was 2,4‰) the level of glycogen in tissues, beta-hydroxybutyrate in blood serum did not change and this could be associated with an acute death before energy reserves are utilized. In 2 cases (alcohol was absent) the level of glycogen in the tissues was normal, there were no histological signs of hypothermia and the concentration of beta-hydroxybutyrate in one case did not differ from the normal level and in the other case it was increased. In 4 cases (alcohol was absent in two cases and it concentration was 2,3‰ and 2,4‰ in two other cases) the glycogen level was low, the concentration of beta-hydroxybutyrate was above normal and there were no histological signs of hypothermia. Thus, abnormalities of the tissue glycogen content and beta-hydroxybutyric acid concentration must be interpreted carefully, as they do not allow the diagnosis to be categorically excluded or confirmed. Preexisting metabolic dysfunctions, blood ethanol levels, duration of death may contribute to the increasing and decreasing of glycogen content and beta-hydroxybutyric acid concentration.

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Suicide Ideation and Hopelessness Among Substance Users in Probation System

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Abstract

As in the whole world, substance use behavior is also increasing in Turkey [1, 2]. This situation also causes some psychosocial problems. One of these problems is suicidal ideation and hopelessness are high in substance abusers [3, 4]. It is known that there is a reciprocal relationship between substance use behavior and suicide [5]. This situation is an important obstacle to the prevention and intervention of substance abuse. At this point it is important to determine the risk factors that cause hoplessness and suicide ideation. Therefore, the aim of this study is to determine the relationship between various psychosocial characteristics and suicidal ideation and hopelessness of substance users. For this purpose, it is conducted with 256 male substance users by purposive sampling method in Ankara Probation Service. The variables were gathered from Personal Data Form, Hopelessness Scale and Suicide Ideation Scale. The difference between psychosocial variables and hopelessness and suicidal ideation was analyzed by independent-sample t test and Anova. According to the results, it was found that economic insufficiency, psychiatric diagnosis, substance use in the early ages, substance use in the family and social environment, exposure to violence in childhood or adolescence cause to increase hopelessness and suicidal ideation (Table 1). In the light of the results, risk factors should be analyzed in substance users who have hopeless feelings and suicidal ideations, and multifactorial based psychosocial studies should be done.

Key words: Substance use, suicide ideation, hopelessness.

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Table 1. Independent-sample t test and Anova results for group differences.

		Hopelessness Scale				**Suicide Ideation Scale**		
Variables		N	M/Sd	**t/f**	**Post hoc**	**M/Sd**	**t/f**	**Post hoc**
Regular work	Yes	168	19.52/5.62	-4.34^{***}		12.59/5.62	-3.23^{***}	
	No	88	22.81/6.08			14.95/6.06		
Income	Non-income^{a}	47	22.60/6.09			15.36/5.62		
	100-1000 Tl (24-230€)^{b}	70	21.98/5.66			13.94/6.11		
	1001-2000 Tl (231-461€)^{c}	75	19.89/5.06	3.73^{**}	a>d	12.21/4.28	3.10^{**}	a>c,f c>f
	2001-3000 Tl (462-691€)^{d}	31	18.87/4.86			13.03/5.48		
	3001-4000 Tl (692-922€)^{e}	16	18.69/8.56			12.50/6.71		
	923 € and over^{f}	10	17.20/5.37			9.90/1.73		
Psychiatric diagnosis	Yes	50	22.38/6.11	2.20^{**}		15.74/5.71		3.35^{**}
	No	205	20.30/5.91			12.82/5.43		
Onset age of substance use	Before 18 years^{a}	139	27.71/6.03			13.96/5.78		
	18-23 years^{b}	62	19.79/5.57			12.97/5.19		
	24-29years^{c}	24	18.46/5.52	3.45^{*}	a>e	12.41/6.08	No significant	
	30-35years ^{d}	13	17.15/5.87			11.07/4.31		
	36 and over years^{e}	4	21.63/5.98			14.25/5.60		
Subtance use in family member	Yes	96	21.64/6.48	2.16^{*}		13.56/15.75	No significant	
	No	156	20.66/5.69			13.13/5.42		
Substance use in friends	Yes	214	21.88/5.99	2.49^{**}		13.67/5.71	No significant	
	No	43	18.62/5.58			12.13/4.86		
Exposure to violent	Yes	93	22.89, 6.27	4.63^{***}		15.40, 6.41	4.42^{***}	
	No	164	19.43, 5.45			12.29, 4.76		

N = 256, $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Toward Actionable Intelligence in Cybersecurity Forensic Investigation

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ABSTRACT

Digital forensic science provides scientifically proven methods that can be used to identify, collect, acquire and preserve digital evidence. Setting an absolute standard that dictates “work from an exact copy of the original data” is dangerous from the 2001 first Digital Forensic Research Workshop in conducting cybersecurity forensic investigation [1]. It has ignored the urgent need for first responders to find actionable intelligence immediately at crime scene. Law enforcement agencies need to explore the crime scene, gather digital data in different devices, and find actionable intelligence immediately. Collecting volatile/non-volatile information helps them respond quickly from artifact behavioral analysis. An experiential observation from penetration test will be conducted to pursue investigation leads. This study takes an experiential observation from penetration test and aims at the relevant activities at crime scene. A digital evidence governance strategy is proposed in Table 1 to improve the investigation process.

Keywords: Crime Scene Investigation, Cybersecurity Forensic Investigation, Penetration Test

Acknowledgments: This research was partially supported by the Ministry of Science and Technology of the Republic of China under the Grants MOST 106-2221-E-015-002.

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Table 1. Digital evidence governance strategy to improve the cybersecurity forensic investigation.

Process	Body of Knowledge	Type	Sample
Identify	Sources of Digital Evidence	Host	records, files, folders , and logs
		Network	wireshark, e-detective, and diamond a-packetman
	Artifacts	External	the internet, social media, and criminal networks
		Internal	systems, personnel, and communications
Collect	Tools	Native	net, ps, ipconfig, netstat –ano, regedit and so on.
		Third-Party	nmap, tcpview, autoruns, ostriage, and so on.
	Memory	Volatile	registers, cache, routing table, ARP cache, process table, and kernel statistics
		Non-volatile	disk, remote logging, monitoring data, physical configuration, and archival media
Acquire	Location	Scene	actionable intelligence
		Lab	forensic integrity
Preserve	Evidence Collection	Automated	alters-based logging and sweep collection
		Manual	native and third-party
	Forensically Sound	Reliability and Trustworthiness	work from an exact copy of the original data
		Relevance and Sufficiency	find actionable intelligence immediately at crime scene

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Indicative of Violence in Homicidal Women

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ABSTRACT

The purpose of the multidisciplinary investigation was to analyze the participation of violence in homicidal women [2], from the criminological aspects; through the field work done at the Preventive and Female Rehabilitation Center of Puente Grande, Jalisco, Mexico (2008-2014).

In methodology, criminological, sociological and law research methods were handled; with interviews with female inmates accused of homicide; including their life history, using a sample of 25%, and analyzing the psychobiological and socio-legal variables.

In the discussion and results, there is influence of psychobiological and social aspects in the violent behavior of women, and who have had a life history impregnated with violence, mainly by the family, pattern [2] which is repeated with children and / or against those who come to affect them, feeling threatened in their integrity. In this complex analysis there are several indicators and external and internal agents that encourage women to apply violence. This entails an interfactorial and multifactorial relationship between related causalities, as in the study of criminal victimology.

It is concluded that violent acts carried out by homicides come from a combination of multifactorial aspects, in addition to the change of role from victim to victim, in 90% of cases, which brings with it psychological and social problems in the behavior of the homicides, being added in some of them the mental illnesses and with a high degree of aggressiveness.

Keywords: Violence, homicides, victimology, life history, indicatives.

Acknowledgments: Thanks to the authorities of the Penitentiary Centers of Puente Grande, Jalisco, Mexico, to the Attorney General of Human Rights of the State of Jalisco, to collaborators and family for the support.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Death by adjustable plastic clamp: a singular case of suicide ligature strangulation

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Abstract

Asphyxia encompass all conditions caused by the failure of cells to receive or utilize oxygen. They are common in forensic practice. Strangulation is a form of asphyxia characterized by closure of the blood vessels and/or air passages of the neck as a result of external pressure to the neck. In ligature strangulation the pressure on the neck is applied by a constricting band tightened by a force other than the gravitational weight. Etiology is often problematic, as suicide by ligature is suggestive for homicide. Investigation of the scene is paramount as external and internal findings may provide clues to better understand the circumstances involved.

The authors present a singular case of a 48 years old man, found by his wife and child at home, lying in ventral decubitus, with an adjustable plastic clamp around the neck which was cut off by the victim's wife. Technical police processed and photographed the scene, finding an alleged suicide note near the body. Interviewing the family revealed a social context of unemployment and a clinical setting of depression.

Examination of the clothes revealed no sign of trauma. External examination of the body revealed an ecchymotic mask and conjunctival petechiae. On the neck there were two shallow ligature marks, below the thyroid cartilage, which intersected one another with an incomplete furrow on the back with an hemorrhagic crest measuring 0,7 cm of width. Bruising of the posterior aspect of the parietal region was observed as well as two bruises on the upper left arm. Although the first's location was not in accordance with the position in which the body was found, due to scarce circumstantial information, it could not be excluded that the lesion could have resulted from a fall to the ground.

Internal examination showed a condensation line along the neck in correlation with the external ligature mark, associated with infiltration of the neck muscles. No fracture on the hyoid bone or thyroid cartilages was identified and there was visceral congestion of the lungs, kidneys and larynx. Auxiliary exams revealed 0,18 g/L of alcohol and anti-depressive in therapeutic/ toxic dosage.

To better examine and interpret the characteristics of the ligature marks on the neck, the medical team requested the plastic clamp (initially not recovered). Although atypical in number and conformation, after presented with the constricting element, and considering the ventral decubitus position, compatibility was determined between the two.

Considering the information provided by the police, the autopsy findings and the results of the complementary exams, the cause of death was due to asphyxia caused by ligature strangling in a likely suicide setting. With this presentation the authors intend to emphasize the importance of good cooperation between the police and the medical experts who conducted the autopsy

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Potential pharmacogenetic inference in post-mortem investigation

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ABSTRACT

The potential strength of pharmacogenetics in medico-legal context has been reported, even if the application in the medico-legal casework and the courtroom transposition need more scientific studies. When the cause of death (CoD) and/or the manner of death (MoD) are unclear or the toxicological results are difficult to explain, the pharmacogenetic investigation can be performed and the results need to be evidence-based interpreted [1]. Inter-individual variability in drug response derives from genetic polymorphisms in drug metabolizing enzymes which affect their function and lead to altered drug responses to a very large extent. In this respect, polymorphisms of the cytochrome P450 (CYP) enzymes play a major role being responsible for the metabolism of 70-80% of all phase I metabolism of clinically used drugs and participating in the metabolism of several xenobiotics [2]. The genetic bases of the polymorphism are single nucleotide polymorphisms, insertions/deletions and gene copy number variations and CYP2C9, CYP2C19 and CYP2D6 are the most polymorphic enzymes which mediate about 40% of P450-mediated drug metabolism [3]. The most extensively studied is the highly polymorphic CYP2D6 gene with up to date 109 allele variants permits to distinguish individuals in ultra-rapid metabolizers (gUM) normal metabolizers (gNM), intermediate metabolizers (gIM), and poor metabolizers (gPM) [4]. The extreme phenotypes (gPM and gUM) are the most important as they could lead to fatal adverse drug reactions or metabolic toxicity.

Here we describe cases of drug addiction and fatal drug intoxication in which pharmacogenetic testing was applied for the interpretation of past organ failure and post-mortem toxicology results.

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Post mortem eye temperature measurement in time of death estimation – the presentation of new series of cases with exactly known time of death

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Available Online: 15 December 2017

ABSTRACT

Estimation of the TOD is an important issue for forensic pathologist examining the body at death scene. The TOD estimation methods based on post mortem changes such as hypostasis, rigor mortis, rectal temperature, or different supra-vital reactions or even biochemistry, molecular biology etc. are still of not satisfactory precision. In the current study single (20 patients) or double (within 1 hour interval in 10 patients) eyeball and rectal temperature measurements were taken in patients who died in Medical University of Gdansk Hospital Intensive Care Unit. The actual TOD in each patient was exactly known and the body temperature was recorded shortly later (between 0.5 h to 3.5 h). The temperature was measured using pin probes connected to a high precision electronic thermometer. The measured eye temperatures ranged from 29,7 to 33,6 °C. Ambient temperatures in all cases was stable (22 °C) what corresponded to usual room temperature. TOD was calculated using a formula based on Newton's law of cooling previously successfully applied in comprehensive studies on pigs and recent studies on humans:

$$t = -\frac{\ln\left(\frac{T - T_a}{T_0 - T_a}\right)}{kc}$$

where t is the time since death, T is the temperature of the body site, Ta is the ambient temperature (constant: 22 °C in the present study), T0 is the initial human eye temperature (assumed to be 35 °C), kc is a first order cooling rate constant. Thanks to stable ambient temperature and knowledge of the exact time of death the actual study allowed to adjust the mean value of kc= -0.2 h-1 in comparison to the recent studies. Thanks to both the significantly faster postmortem decrease of eye temperature and the residual or lack of plateau effect in the eye, also no influence of body mass, TOD in the human death cases using eq. 1 could be estimated with quite a good accuracy. The maximum TOD estimation error during the post mortem intervals up to 3.5 h was slightly higher than 1 h min in 4 cases among 30, while for the rest of 27 cases it was less than 1 h, while mean error for all 30 cases was ± 24 min. The actual results from 30 new cases with exactly known TOD show that the presented method of TOD estimation may be of satisfactory accuracy in the early postmortem period, particularly when applied to bodies found at room temperature and in normal environmental conditions (still air, normal humidity).

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A semantic platform for radicalization analysis in social streams

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ABSTRACT

During last years, terrorist organizations make use and exploit widely the social media networks to promote their ideas and recruit new foreign fighters. Syria is probably the first conflict in which many Western fighters have been documenting their involvement in conflict in real-time, and where – in turn – social media represents an essential source of information and inspiration to them. The early detection of foreign fighters' recruitment by terrorist groups on social media has become a key point for governments and researchers to counter this phenomenon. In this paper, we present Safapp, a semantic platform to support early detection of foreign fighters' recruitment by terrorist groups in analysing both the literature and the messages posted on social networks. In Safapp, we use syntactic and semantic technologies to support the building of a knowledge base which can then be used to analyse data extracted from social network in the context of research in sociology or in intelligence. The whole Safapp system consists of five main components:

- An advanced, big-data compliant data collector by adapting open source crawlers to fit the need of the project
- A categoriser to filter out irrelevant messages
- A syntactic engine to detect Foreign Fighters and in general terrorism related information
- A semantic engine to complement the detection of Foreign Fighters and in general terrorism related information
- A graphical user interface allowing users to explore, discover and visualize the enriched and analyzed data.

The different level make use of natural language processing and machine learning to detect named entities, basic relevant concepts and categorize noisy messages. Safapp also provides a novel navigation design of the enriched and analyzed data dealing with recruitment and radicalization on Twitter and their evolution in time.

Acknowledgements: This work is Co-funded by the Internal Security Fund of the European Union

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

The paradox of non-motivated + intentional homicide. A case in forensic psychiatry

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ABSTRACT

The motivation for violence against other persons, and homicide in particular, can be classified as reactive or instrumental [1]. Reactive homicide explains a behavior emotionally driven by fear, anger or rage and less often by disgust or sadness. On the other hand, an instrumentally motivated homicide indicates that the deadly behavior is a mean to achieve a different purpose, not always evident for crime analysts, but in general clear in the mind of the perpetrator [2]. The characteristics of the crime scene (CS) are, in general, in accordance with the main motivation. 'Disorganized' CS are more likely to find when reactive homicide occurs, when emotions overwhelmed the perpetrators and overflow to the physical environment itself. Conversely, 'organized' CS are more frequent among instrumental homicide [3] indicating that the perpetrator have prepared him/herself to a certain extent (i.e. shows premeditation). However, those who work as investigators have the experience that real homicides do not fit such academic dichotomies. Very often organized and disorganized elements are found on the same CS. Similarly, forensic psychiatrists/psychologists have to deal with offenders that show a mix of reactive and instrumental motives and behaviors, that sometimes intricate with preexistent severe mental disorder or drug abuse. Seldom, we have to deal with criminal behavior that does not fit any taxonomy or classification system. In such rare cases, homicide is intentional but no underlying motivation can be unmistakably identified. The case we propose to analyze in this paper follows no pattern, no rationality. The crime dynamic and perpetrator's behavior afterwards are bizarre, as are the explanations provided by himself during interrogation and forensic expert assessment. Nguyen was only 17 years old when one day he came to school as any other normal day. He had in mind no intention to assault or find a victim. But he had a jackknife in his pocket which he used to kill an unknown woman in an unknown (for him) place. Three times he stabbed her, in the neck and chest, in an unlikely crime. Afterwards he cleaned the knife in a closer creek and walked serenely until the local police headquarters where he turned himself in. The total lack of any emotional experience not only in relation to the crime committed or the victim but also in relation to himself, to his social and physical environment, and to his life as a whole seem what best can explain Nguyen's conduct. The crime is not susceptible of being classified as instrumental or reactive and the CS could not be characterized as organized nor as disorganized. We will provide arguments for why Nguyen should not be considered a severe mentally disordered offender but rather an authentic case of the "mask of sanity" [4], that situates him in an extreme of the psychopathic spectrum.

Ackowledgements: My acknowledgements are for Mr. Robert Schött (Sundsvall district attorney) who gave me the authorization to read the interviews and forensic assessments, which are confidential information, and also for the personnel of Sundsvall Tingsrätt who friendly provided me a place and everything I needed while I was analyzing all the documents and materials.

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Implication of psychiatric and psychologic assessment in forensic observation

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ABSTRACT

Intoxication with ethanol is widely recognized as a risk factor for a crime perpetrating. In nearly 70 % of delinquents committed murder or a serious injury of a victim's body consumed alcohol 24 hours before a violent act. The study evaluates the relationship between the results of psychological examination and of alcohol intoxicated perpetrators of a homicide or a serious bodily harm in relation to assessment of diminished criminal responsibility by court experts in psychiatry field. The examined group consisted of 90 offenders in the public prosecutor investigation. The perpetrators were referred to the six weeks forensic observation. (suspected for homicide, attempted homicide and grievous bodily harm). The medical records gathered during conducted observation were retrospectively assessed. Unambiguous forensic reports of two courts witnesses were also analyzed. All respondents had undergone an extensive, psychological investigation. The level of intellect was determined by means of Wechsler Test, scored in full IQ scale and ranged from 53 to 130 points. In 62% of evaluated individuals, psychologists could not excluded organic lesions the of the central nervous system. Examinations used in the assessment of a brain organic damage were Benton and L. Bender tests. The majority of perpetrators manifested various personality disorders. The most commonly used tests were MMPI or Diagnostic DKO Questionnaire. In 30% cases organic personality disorders were diagnosed. The Antisocial personality disorder was examined in 31% of individuals. Other personality disorders were observed in 26% of evaluated Only in 11% of the defendants no personality disorder was examined. The results of psychological investigations have significantly correlated with the ruling of diminished criminal responsibility in the assessment of experts in psychiatry field. Diminished responsibility was evaluated mainly in cases of the offenders who presented clinical symptoms of organic personality disorders,. The full criminal responsibility more frequently was assessed in delinquents with diagnosis of personality disorders included antisocial personality disorder. The Study found that none of the offenders was assessed as insane. In 15% of the respondents the diminished responsibility was evaluated. Influence of alcohol at the time of the act, was evaluated as additional circumstances of the criminal acts.

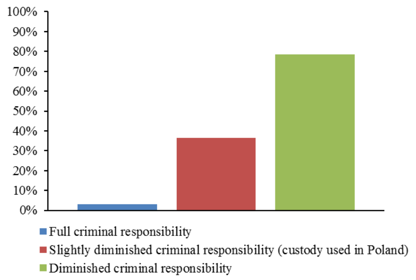

Figure 1. Diagnosis of the organic brain disorder evaluated in psychological examination

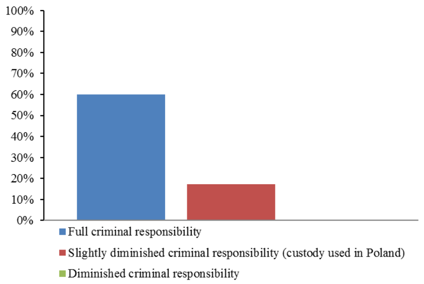

Figure 2. Diagnosis of the antisocial personality disorder evaluated in psychological examination

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Table 1. . Description of population - quantitative variables IQ assessment in the psychological examination

Variable	N*	Average	Median	Minimum	Maximum	SD
IQ full scale	90	90,57	90	53	130	15,36
IQ verbal scale	72	89,65	90	43	124	15,37
IQ non-verbal scale	72	91,26	91,5	49	137	17,29

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

The use of Kalashnikov (AK-47) in 'Ndrangheta murders: The firearm of the clan

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ABSTRACT

'Ndrangheta is a mafia criminal organization, hailing from Calabria, Italy. This organization is able to use any kind of weapon and the choise depends on the type of murder to commit. So, even bazooka have been used when the victims, judges or rival mafia clan boss, travelled by armored cars. Kalashnikov is not only used "normally" to commit mafia ambushes, but often it has been found carbonized with the car used by killers. This act confirm that mafia clan have available vast arsenals of weapons and it is a demonstration of what this organization is able to do. Gunshot wounds cause significant mortality and morbidity. The analysis of the features of injuries makes it possible to establish which kind of weapon has been used. The AK-47 is a selective-fire, gas-operated assault rifle and it uses a long stroke gas system. In order to shoot, who uses a AK-47, inserts a loaded magazine, pulls back and releases the charging handle, and then pulls the trigger. It can be semi-automatic, when the firearm fires only once, or full-automatic, if the rifle continues to fire automatically and cyclically fresh rounds into the chamber. AK-47 rifle bullet injuries present with uncharacteristically large entry wounds and cause complex structural injuries. The consequent trajectory is difficult to predict making regional examination and radiological investigations. Bullets may be retained, leaving no exit wound. Kalashnikov is not a frequent weapon, so the wounds are not so common to see in the forensic practice. But, in 'ndrangheta homicides, this firearm is preferred for its high harmful power that ensure a murder "without mistakes" and with devastating consequences on the shot body. Case Report: We reported a case of young man killed by a Kalashnikov. The corpse was in the prone position. Six holes of a firearm on the body were observed; particularly to the back, chest and left arm. The lesion situated on the chest was the size of 3x2 cm. At the time of inspection the plan metric for ballistic calculations was performed in the following manner: shooting cameras, integrated with the satellite images of the crime scene extrapolated from the website http://www.bing.com/maps/; identification of the location of the discovery of the corpse throughout the use of cadastral maps; measurement of the building and the land in front of the building in the crime scene; pads planimetric and 3D views of the crime scene; shoe-pads and biological agents; evidence collection of shells and cartridges found on the ground; location of shell casings and measurement of distances from the site of the discovery of the corpse. The measurements were used by the ballistic engineer to make the vector calculations. On the scene the ballistic elements were collected. An external examination of the body was carried out, and subsequently a CTMS and autopsy were performed. On the corpse was found a single fatal blow whose trajectory was: 1-rupture of the dorsal vertebrae, 2-laceration of the left lung with hemorrhage, 3-outbreak of the heart and pericardium and massive hemothorax. The ballistic calculations have allowed us to establish that the subject was facing the shooter at first and then, in an attempt to escape, he was back than killer. In the case presented the speed and violence of this firearms have caused an explosion of the heart. These data show the potentially devastating effects of this weapon. Often, this harmful effects make the shooting dynamics reconstruction difficult. For this reason we underline the importance of multidisciplinary approach in assessment of the murders in these cases. Only a careful evaluation within the inspection and the performance of vector calculations on the crime scene and the corpse allows a reconstruction of the murder and becomes a scientific evidence in the court for obtaining a correct reconstruction of the events.

Keywords: Forensic Science, Kalashnikov, Multidisciplinary Approach

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Linguist markers to early detection of radicalization in Social Networks

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ABSTRACT

Nowadays, social networks are essential communication tools that produce a large amount of information about their users and their interactions. Spreading propaganda in digital environments is a good way for various extremist groups who want to reach out with their messages, and it is considered to be an important part of the terrorist group Islamic State (IS) success in recruiting supporters from all over the world. Although propaganda is not the sole cause of radicalisation or recruitment to violent extremist ideologies, interactions on social networks can be an important component of a radicalization process due to its easy accessibility and the ability to capture and retain an individual's interest. Even though it is not clear what role Internet and social media plays in radicalization, some previous studies have focused on measuring the risk for individuals to radicalization [1,2] and the possibility to detect individuals or groups that engage in violent extremism [3]. In this work, we focus on identifying a set of linguistic indicators that can be used to measure frustration, the perception of discrimination, and the declaration of negative and positive ideas about the Western society and Violent extremism respectively. The indicators have been tested on three different datasets: tweets by pro-ISIS users, tweets from users flagged as radicals by the Anonymous collective and a random sample of tweets gathered from the public Twitter stream.

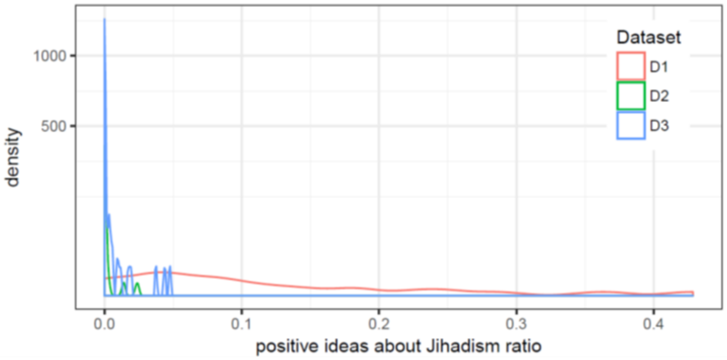

Figure 1. Density distribution of the ratio of tweets expressing positive ideas about Jihadism according to the studied indicators and their respective metrics .

Ackowledgements: This work has been supported by RiskTrack (JUST-2015-JCOO-AG-7231809) and EphemeCH (TIN2014-56494-C4-4-P) projects. The contents of this publication are the sole responsibility of their authors and can in no way be taken to reflect the views of the European Commission.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

The importance of Online Social Networks on the radicalisation risk assessment

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Available Online: 15 December 2017

ABSTRACT

With the growth of the Islamic terrorism and groups like Islamic State or Al Qaeda, academics have focused on detecting risk factors to understand the radicalisation phenomena and, thus, preventing it. Even though there is not a single profile of an Islamic radical, most of the authors point to several conditions that are partially shared by most of those radicals. Moreover, the development of the Online Social Networks, such as Facebook or Twitter, along with the Internet, has created a new field for the radicals to start their radicalisation paths, but also a new chance to detect the behavioral changes they present there. With this change on the rules in mind, this paper focuses on assessing the behavioral traces that can represent a sign that a person is becoming radicalised on the Online Social Networks. Both theoretical [1] and empirical information [2, 3] will be take into account in order to create a final report of the radicalisation risk factors and their indicators presented on the Online Social Networks

Table 1. Personal risk factors.

Generation of migrant	Educational level	Age	Mental health	Attitude towards politics	Religious orientation	Social isolation
Pro-terrorism attitude	Psychological factors	Criminal record	Social isolation	Exposure to radical propaganda	Economical level	Travel to a conflict zone

Table 2. Group risk factors.

Family conflicts	Perceived discrimination	Sense of belonging to a group	Use of radical rhetoric
Identification with Muslim victims	Relationships with radicals	Presence of a charismatic leader	

Ackowledgements: This work has been supported by RiskTrack (JUST-2015- JCOO-AG- 7231809) and EphemeCH (TIN2014- 56494-C4- 4-P) projects. The contents of this publication are the sole responsibility of their authors and can in no way be taken to reflect the views of the European Commission.

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Representation of information in Social Network to Perform Community Finding Tasks

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Abstract

The unstoppable growth that Social Networks (SN) have suffered in the last years, has produced that the data stored in those networks grows exponentially. This data appears from the information that the users provide in their corresponding profile, the different connections that they stablished while they are using the SN, and also due to the different interactions that the user perform within the SN. All this data has become in a great opportunity to extract information from the SN and also from the users. One of the most typical information that can be extracted from this data is the different groups, or clusters, of users. The main idea is to gather users in one or more groups in such a way users belonging to the same group are similar, whereas there are several differences among the users of the other groups. This problem is commonly known as *Community Finding Problems*, and the different groups of users are called "*communities*" [1]. The data used to perform the community detection task is critical because it will affect to the quality of the communities found by the algorithms. In this way, it is possible to detect the different communities based on data extracted from the network (such as relation between users), or based on the information provided by the users in their profiles [1]. But it is also possible to compute other metrics related to the users behaviour in order to define the different communities [2, 3, 4]. The goal of this paper is to analyse the different approaches that can be used to perform the *Community Finding Tasks* taking into account the different types of data available in the most popular Social Network.

Ackowledgements: This work has been supported by RiskTrack (JUST-2015-JCOO-AG-7231809) and EphemeCH (TIN2014-56494-C4-4-P) projects. The contents of this publication are the sole responsibility of their authors and can in no way be taken to reflect the views of the European Commission.

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Spinal cord injury in Penal Law – a singular case of serious bodily harm

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Available Online: 15 December 2017

ABSTRACT

Spinal cord injury is an insult to the spinal cord resulting in a change, temporary or permanent, in the cord's normal motor, sensory or autonomic function, resulting in usually permanent and often devastating neurologic deficits and disability [1]. Epidemiologically is frequently associated with road traffic accidents, affecting mostly young males [2, 3]. In Portugal Penal Law, bodily harm is divided in simple and serious based on four points regarding injury to the body itself and its function from the moment of the event and its after effects. In Forensic Clinic, the vast majority of cases regard simple bodily harm injury; nevertheless it is of utmost importance to recognize and describe to the court complex cases indicating the extent of injuries and the repercussions it brought and bring at the moment of our evaluation [4].

The authors present a case of a young male (29 years old), salesman, victim of motorbike accident of which resulted spinal cord injury due to comminuted fracture T7-T8, multiple rib fracture and kidney, spleen and genital tract trauma. Hypotension and agitation at the site, reverted with IV fluid, blood transfusion, morphine and oxygen. At admission without muscular strength and sensitive level T6. Submitted to surgery T7-T8, without functional gain. Transferred to rehabilitation with progressive recovery, regaining static and dynamic balance of the trunk. Medicated for muscular spasm on lower limbs and antibiotics due to repetitive urinary tract infection. Follow up in Neurosurgery, Physical and Rehabilitation Medicine and Urology (due to erectile and ejaculatory dysfunction). At the discharge date (283 days after the event) the patient presented with a paraplegia ASIA A T5 with no muscular strength on lower limbs (moved in a wheel chair), without voluntary anal sphincter contraction and suprapubic probe. Several auxiliary items for daily activities and medication were prescribed and routine check-ups were scheduled. From a medico-legal perspective, despite further follow-up the injuries, the neuromotor condition is fixed, therefore some conclusion can be elaborated. Regarding Portugal Penal law this case presents the characteristics of a serious bodily injury situation as the lesions were severe, causing a permanent disfiguration and affecting its ability to use the body, to perform its job and have sexual fruition. The characteristics surrounding the event, mainly in the emergency room the patient was in a life threatening situation. As for permanent help for the future the patient will need technical, medical and 3rd person assistance to cope with the after effects (as well as an eventual Urology surgery) to improve his quality of life.

In conclusion, it is vital for the forensic doctor to be prepared to analyze in a medical point of view but also in the scope of the area of the Law involved the case presented, articulating with other areas of expertise so that it may transmit to the court with exemption, impartiality and objective the consequences of bodily harm.

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Post-mortem civil law evaluation – a challenging approach regarding two cases

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ABSTRACT

According to Civil Law in Portugal, whom who is obliged to repair an injury must do so in order to restitute the situation previously verified being compulsory the indemnity of the injured party. Damage may classified in temporary or permanent as well as pecuniary and non-pecuniary [1,2]. Civil law evaluations are frequently performed to victims of road traffic accident regarding monetary compensation. The authors present two cases on which this evaluation was performed after the victim has deceased, with its inherent challenging approach.

Case 1 – male, 57 years old, victim of road traffic accident in 2008 which resulted amputation left leg and deformity on the right ankle. Treated by Orthopedics, Pshysical and Rehabilitation Medicine and Psychiatry (depression). Follow up in clinical services of the insurance company, submitted to several surgeries. In 2012 was diagnosed with retroperitoneal leiomyosarcoma with metastases. The medical experts were asked if a correlation between the event and the cause of death could be made.

Case 2 – male, 43 years old, victim of road traffic accident in 2010, with serious brain injury and skull fracture (Glasgow scale 6 at admission). Conservative neurosurgery follow-up followed by hospital discharge two months later to rehabilitation clinic with reasonable functional gain. Evaluated two years later regarding Penal Law with severe cognitive handicap (frontal syndrome) as well as motor dysfunction of left arm. The medical experts were asked about Civil repercussions of the event, namely if the victim (meanwhile deceased in 2013) died due to after effects of the accident.

In both cases a Civil report was made regarding the injuries and consequences due to the accident based on Portugal Civil Law. On case 1, based on clinical records no clinical correlation was established between the accident and the tumoral lesion. On case 2 the last written clinical record dated two weeks before the date of death. For that reason a natural cause of dead was not possible to be excluded and medical experts had not information do correlate the after effects and the cause of dead. Despite the specificity of Legal Medicine Civil evaluation, other clinics should be aware of the importance to document clinical information as it may be important even after the patient life has ceased.

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Forensic Science and Law Discourse: On the Linguistic Difficulties for Translators and Interpreters

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ABSTRACT

Forensic Science (or Forensics) holds various fields of science (Psychology, Pathology, Odontology, Toxicology, Digital Forensics, to cite a few) involved in solving crimes and offences at any stage in criminal proceedings and researches. On the other hand, Forensic Law is a legal branch that involves issues related to forensic techniques in a justice system (being thus linked to Criminology, Criminal Law, and Civil Law). The intersection of Science and Law contributes to finding out the truth of a case, either criminal or civil, and they both have undergone dramatic progress in recent years. The aforementioned intersection provides specialists with a considerable amount of documents, either electronic or paperback…and they often need to be translated or interpreted into other languages. The high level of technical terms, complex nouns and phraseology, hamper their translation, interpreting, and proofreading services. In this paper we will provide the reader with the main linguistic features of Forensic Science and Law Discourse, matched with source texts and target texts. By means of the use of original Forensic documents, we will provide translators and interpreters with strategies and alternatives for facing their translation and proofreading

Keywords: Forensic Science; Forensic Law; Linguistic difficulties; Translation; Proofreading

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Trends in diagnostic of fatal traumatic brain injuries

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Available Online: 15 December 2017

ABSTRACT

Traumatic brain injuries (TBI) affect people of all ages and genders and contribute to a substantial number of deaths or cases of long-term disability. Therefore, such injuries are an important part in daily routine of legal medicine. The morphological and biochemical demonstration of marker profiles after TBI is of considerable interest in legal medicine for determining the causes and mechanisms of traumatic death. Whenever possible, a precise estimation of the survival time after TBI is necessary to solve forensic questions.

A lot of our studies describe the time course of expression of different markers of the central nervous system via histological, immunohistochemical, immunofluorescence, biochemical and molecular genetic methods, which may be used for estimation of the wound age after TBI and to get further insight in the molecular pathways of so-called secondary injury to the brain with inflammatory response, brain swelling, hypoxia and oxidative stress changes over hours, days to months.

In an overview the authors will present useful signs to estimate the vitality of fatal head impacts and to differentiate short and longer survival times of TBI. They will demonstrate the damage of neuronal and glial cells by time-dependent changes of necrosis, apoptosis, receptor regulation and proliferation. Our research findings emphasize the potential of detailed forensic examinations in cases of suspected TBI

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SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE IN TRANSLATIONAL FORENSICS (FORENSICS 2017)

Global Responsibility: The Role We Play in Developing Forensic Science

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Abstract

Forensic science works to support justice. It doesn't belong to just one nation, but is critical to the global community. In the United States, we are fortunate to have a strong understanding and history of utilizing investigative principles and emerging technologies to serve our communities. It's time our scientists reach out to work with global laboratories to share our best practices and knowledge.

Through our work with the United States Department of State, the National Forensic Science Technology Center is seeing firsthand the discrepancies in forensic science practice that need to be addressed. By creating relationships between laboratories, in sister cities or via similar programs, we can encourage discussions about improving forensic science practice worldwide.

Our partnership with ANAB and ANZPPA allows us to use our two decades of experience in helping international laboratories acheive accreditation. Most recently, we've joined forces with Florida International University. Our clients, current and future, will now not only have our talent pool, but the entire staff of American's fourth largest university, providing unparalleled access to top tier subject matter experts.

It's our responsibility to share the impact forensic science can play on a global scale.

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Nanotechnology: Identification of early time passed since death

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Available Online: 15 December 2017

ABSTRACT

The breakthroughs of fluorescent nanosensors have paved the way for biomedical research. Moreover, the reported sensibility and specificity of the chemo- and biosensors opened the door to more investigations which will introduce new applications either by optimization of already developed sensor or discovery of new ones. Most of nano researches are directed towards medical applications, although it could also be introduced towards medico-legal applications. In this direction, new nanosensor was designed and examined to detect postmortem changes in blood. The newly designed sensor could be used as a marker to detect changes happened in blood postmortemly.

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A case of homicide by captive-bolt gunshot

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ABSTRACT

Captive-bolt guns are used as weapons for human slaughtering of animals in meat industry and should be paced against the animal's forehead to induce immediate unconsciousness before slaughtering livestock. They consist of a simple cilindrical metal tube (barrel) with a metal bolt placed in the centre (approximately 7-15 cm long and 1-1.5 cm wide). The bolt is actuated by a trigger pull and is propelled forward by compressed air or by the discharge of a blank powder gun cartridge. Violent death inflicted by captive-bolt guns are rarely reported in forensic pathology and are predominantely suicidal, while accidents and homicides are unusual events. We have observed a case of homicide with head injury by a captive bolt type of slaughterer's gun, brand mark "Humanitas"(Fig.1). In the occipital region we found a contused wound, oval shaped, with irregular contused edges. The edges were bent inwards with a small blackish-red abrasion collar and underlying gap in the skull with caudocephalic diameter of approximately 1.3 cm, clean edges on the outer table of the bone and slightly flared edges on the inner table (Fig.2). At the bottom of the wound within the cerebellar matter, there were several small fragments of bone and hair, punched inward (Fig.2). We appreciated subarachnoidal bleeding and laceration of meninges with serious encephalic and cerebellar injury, causing death.

Figure 1. Slaughterer's gun "Humanitas"

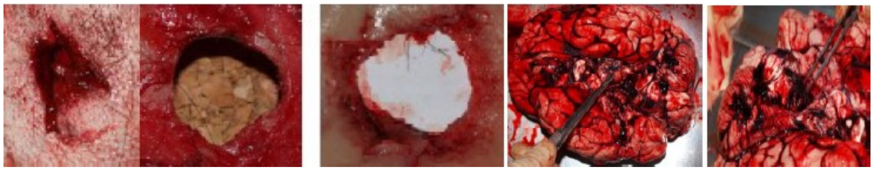

Figure 2. Autopsy findings

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