Volume 8 | Issue 3 | December 2018 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.

Editors-in-Chief

Regional editors

Asia

Australia and New Zealand

Europe

Australia and New Zealand

JOURNAL OF INTEGRATED OMICS

A methodological Journal

SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

ORIGINAL ARTICLES

SPECIAL ISSUE:
SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

The content published in this issue was previously selected by the scientific committee to be presented at I International Caparica Conference on Leishmaniasis, from 29 - 31 October 2018 in Caparica, Almada, Portugal. Only the Proceeding Abstracts with a copyright transfer agreement were reproduced.

I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Caparica – Lisbon, Portugal – 29th-31st October 2018

Commitees

Conference Chairs

***Carlos Lodeiro Espiño*, PhD. FRSC**
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
***José Luis Capelo Martínez*, PhD. FRSC**
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.

Scientific Committee

Carlos Lodeiro Espiño, PhD. FRSC
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
José Luis Capelo Martínez, PhD. FRSC
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
Gilberto Igrejas, PhD
Universidade de Trás-os-Montes e Alto Douro, Portugal.
Hugo Miguel Santos, PhD
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal..
Elisabete Oliveira, PhD
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal..
Patricia A. Curado Q. Dinis Poeta, PhD
Veterinary Science Department, CECAV, University of Trás-os-Montes e Alto Douro, Portugal
Lénea Campino, PhD
Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisbon, Portugal.

Conference Secretariat

José Luis Capelo Martínez, PhD. FRSC
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.
Marta Silva, MSc
LAQV-REQUIMTE, Chemistry Department, Universidade NOVA de Lisboa, Portugal.

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Molecular identification of Leishmania species in pediatric population attended at the National Institute of Health

Aide Sandoval-Juarez [1*], Gloria Minaya-Gómez [1], Nyshon Rojas-Palomino [1], L F Donaires [1].

[1] Instituto Nacional de Salud—Perú.

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis is a parasitic disease that affect majority of people of endemic area mainly to the pediatric population, and is caused by a parasite protozoa of the *Leishmania* genus. In children, the identification of Leishmania species infecting is very important, because it allows establishing criteria and protocols for will prevent the progression of disease to more aggressive clinical forms, as mucocutaneous and diffuse leishmaniasis. Our aim was to identify the *Leishmania* species in samples obtained from 30 children (between 1 and 14 years old), who were attended to the Peruvian National Institute of Health and parasitologically confirmated as American Tegumentary Leishmaniasis. The identifiction of *Leishmania* species, were development by High Resolution Melting Analyzing (HRMA) of the conserved region of the kDNA *Leishmania*. Initially, we extracted parasitic DNA from 17 *Leishmania* spp strains isolated by in vitro Culture and 13 smear Giemsa-stain with positive result by microscopy visualization of amastigotes. Later, we amplified the conserved region of the kDNA *Leishmania* parasitic DNA through PCR-High Resolution Melting. The data was recorded and analyzed by RotorGene Q software and Stata 13.0.v. The Median age of the 30 children was 7.5 years old (Intercuartil range [ICR]: 3-11) and 60% (18/30) were male. The median time of disease was two months (ICR:1-4), 83.3% (25/30) of those evaluated had a single lesion, with an average surface area of 4.8 cm^3. (ICR:0.7-5). The identified species were *Leishmania (Viannia) peruviana*, (40%), *Leishmania (V.) guyanensis* (27%), *Leishmania (V.) braziliensis* (20%), *Leishmania (V.) lainsoni* (10%) and *Leishmania (Leishmania) amazonensis* (3%). We found three cases of mucocutaneous leishmaniasis from Huánuco, Ayacucho and Cusco, that were caused by L. (V.) braziliensis, L. (L.) amazonensis and L. (V.) guyanensis, respectively. Also, our found four cases of Leishmaniasis recidivans of children from La Libertad, Lambayeque and Pasco. In Peru, the information related to Leishmaniasis is inadequate, similar occur with information related to Leishmaniasis in children population that is scarce, because the notification just consider general aspect of the confirmed cases, without adequate disaggregation by age , social status, among other. In other hand, we achieves identify *Leishmania* strains isolated from children as *Leishmania (V.) braziliensis* and *Leishmania (V.) guyanensis* from areas where not been previously reported. We recommend continuing studies of identification of *Leishmania* species in samples from children population that involve a greater number of participants, also develop the validation of *Leishmania* species identification through of HRMA of the conserved region of kDNA in compare with gold standard methods or Cytochrome B gene sequencing, and continuing studies related to dispersion of *Leishmania* species to new endemic areas..

References:

Ruskova L, Raclavsky V., Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 155 (2011) 3 239-52. doi: 10.5507/bp.2011.045

R. Reithinger,J.C. Dujardin., J Clin Microbiol 45 (2007) 21-25. doi: 10.1128/JCM.02029-06

Correspondence: Email - asandoval@ins.gob.pe

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Fluoroquinolones: an ancient antibiotic potentially useful against leishmania

Ana Poveda [1*], Javier Santamaría-Aguirre [1], Lissette Esteves [1], Luis Sebastián Espíndola [2], Gina Loachamin [1], Karen Tufiño [1], Gabriela Morales [1], Eliana Lara [1] and Miguel Ángel Méndez [2].

[1] Instituto de Investigación en Salud Pública y Zoonosis, Facultad de Ciencias Químicas, Universidad Central del Ecuador, Quito; [2] Grupo de Química Computacional y Teórica Universidad San Francisco de Quito.

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis is a largely neglected infection caused by *Leishmania* spp. parasites. The first-line treatment, antimonate meglumine, has a large number of adverse effects, high costs and is developing resistance. New alternatives are mandatory. An interesting target are topoisomerases type II, that conserve the critic homologous serine residue inhibited by fluoroquinolones [1]. They have lower cost and fewer adverse effects. To determine the leishmanicidal effect of fluoroquinolones, a fluorescence method was optimized to determine MIC and IC50 in cultures of *L. mexicana* and *L. braziliensis* promastigotes. Our results show a good leishmanicidal activity of fluoroquinolones, being enrofloxacin the most effective. Protein modelling and docking results are consistent and supports that these enzymes are targets of fluoroquinolones. Leishmania parasites have two genes encoding topoisomerases type II, one located at the nucleus and the other in the mitochondria. Pulse field gel electrophoresis (PFGE) and mitopotential suggest that the mitochondrial enzyme is the main target. Enrofloxacin is ionized in a wide range of pH, limiting its absorption through the biological membranes. This limitation could be solved loading it into liposomal systems such as the transferosomes, ultradeformable nanovesicles. Transferosomes were characterized in terms of size, polydispersity index, zeta potential, entrapment percentage, dissolution profile and physical stability. These nanovehicles enhanced the leishmanicidal activity compared with enrofloxacin in solution, around 15 times. So the nanoencapsulation could be an interesting approach to develop a topic formulation to treat cutaneous leishmaniasis.

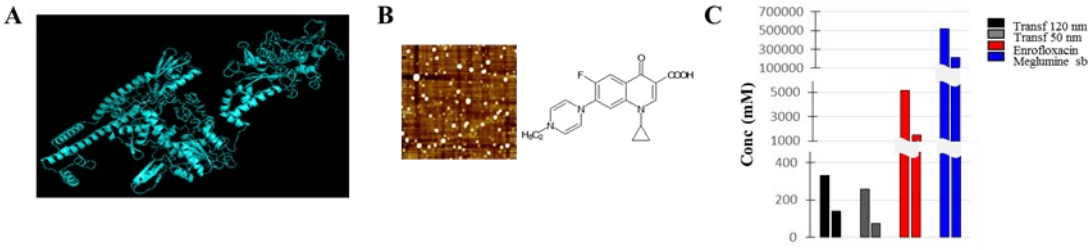

Figure 1 | A. Modelled 3D structure of mtTopoII; B. AFM image of transferosomes and chemical structure of enrofloxacin, C. MIC and IC50 of transferosomes, enrofloxacine and meglumine sb.

Acknowledgments:
This work was financed with funds from project # 21 of the General Directorate of Research and Postgraduate Studies, Universidad Central del Ecuador and with funds from the Academic of Recherche et d Enseignement Supérieur (ARES) of Belgium.

References:

1] Uzcanga, G. et al. . Crit. Rev. Microbiol. 43, (2017) 156-177. doi: 10.1080/1040841X.2016.1188758

Correspondence: Email - apoveda@uce.edu.ec

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Leishmania extracellular antigens properties and future applications

N Santarém [1] , A Cordeiro-da-Silva [1,2*].

[1] Parasite Disease Group, IBMC - Instituto de Biologia Celular e Molecular, Portugal; [2] Faculdade de Farmácia, UP - Universidade do Porto, Portugal.

Available Online: 30 December 2018

ABSTRACT

The use of secretion pathways for effector molecule delivery is an essential part of the millennia struggle between pathogens and their host immune system. Leishmania protozoa are no exception. Recent emphasis has been given to the parasite released extracellular vesicles (EVs) as important agents in this process. Still, these EVs are not alone as players and their significance in the context of other Leishmania secreted antigens is still to be observed. To address this we evaluated the immunological potential of the promastigote exoproteome (EXO) and its associated components EVs and vesicle depleted exoproteome (VDE). We also evaluated the potential use of these antigens for serodiagnosis.

In an air pouch model, a dose-dependent recruitment of immune cells was observed for all the exoproteome components. Interestingly, EVs and VDE induced a different recruitment compared with parasites, attracting significantly more dendritic cells, which remain non-activated at the site of inoculation, and fewer neutrophils. Interestingly in vitro, EVs, VDE, and EXO, as well as the parasites, were able to diminish the response capacity of DCs and macrophages to TLR ligands suggesting that these components interfere with functional aspects of these cells. Overall the secreted antigens conferred a real as they increase the parasite burden in a dose-dependent manner. In the future, these exogenous antigens can be a source of biomarkers for diagnostic and vaccine use.

Correspondence: Email - cordeiro@ibmc.up.pt

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Exploring the effect of a sandfly salivary protein "prime" on the immunogenicity of a complex vaccine formulation containing both vector and parasite-derived antigens

Pedro Cecílio [1, 2, 3*], Begoña Pérez-Cabezas [1, 2], Laura Fernández [4], Javier Moreno [4], Eugenia Carrillo [4], José M. Requena [5], Epifanio Fichera [6], Steven G. Reed [7], Rhea N. Coler [7], Shaden Kamhawi [8], Fabiano Oliveira [8], Jesus G. Valenzuela [8], Luigi Gradoni [9], Reinhard Glueck [6], Gaurav Gupta [6] and Anabela Cordeiro-da-Silva [1, 2, 3].

[1] Parasite Disease group, Instituto de Investigação e Inovação em Saúde (i3S), Universidade do Porto, Porto, Portugal; [2] IBMC - Instituto de Biologia Celular e Molecular, Universidade do Porto, Porto, Portugal; [3] Faculdade de Farmácia da Universidade do Porto, Porto, Portugal; [4] WHO Collaborating Centre for Leishmaniasis, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain; [5] Centro de Biología Molecular Severo Ochoa (CSIC-UAM), Universidad Autónoma de Madrid, Madrid, Spain; [6] Etna Biotech S.R.L, via Vincenzo Lancia, 57 - Zona Industriale Blocco Palma 1, Catania, Italy; [7] Infectious Disease Research Institute (IDRI) , Seattle, WA, USA; [8] Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, NIH, Rockville, MD, USA; [9] Unit of Vector-borne Diseases and International Health, Istituto Superiore di Sanità, Rome, Italy.

Available Online: 30 December 2018

ABSTRACT

Although vaccination is accepted as a potentially effective approach to prevent leishmaniasis, to date there is no vaccine available for human disease. Leishmania parasites are transmitted by sandflies from the genus Lutzomyia and Phlebotomus that, together with metacyclic promastigotes, deposit vector saliva into the host skin during the blood feeding process. Sand fly saliva was shown to be an infection enhancer, and counter-intuitively to confer anti-Leishmania protection upon multiple exposures. Yet, most of the works concerning Leishmania and leishmaniasis have a binomial focus (host-parasite) and disregard the contribution of the vector. Such neglect, in the context of anti-Leishmania vaccination is both a limitation, as highlighted by Peters et al that have shown the loss of protection of a supposedly-good vaccine candidate, when tested in the context of vector-transmitted leishmaniasis; and an extravagance, having in consideration that vector saliva is an untapped source of antigens for Leishmania vaccines.

Having in consideration the trinomial nature of this vector-borne disease, we explored in the pre-clinical context, an innovative vaccine approach for human visceral leishmaniasis consisting of three different recombinant proteins (LJL143 from Lutzomyia longipalpis saliva as the vector-derived (VD) component, and KMP11 and LeishF3+, as parasite-derived (PD) antigens), either free, or formulated in Influenza virosomes, and adjuvanted with GLA-SE, a TLR4 agonist. Interestingly, the immune responses generated against the VD protein were reproducibly more robust than those elicited against leishmanial antigens, and were apparently not caused by immunodominance of the VD antigen.

These results directed our focus to the investigation of the influence of an unusual immunization scheme in the memory responses generated by our complex vaccine candidate. Remarkably, priming with the VD protein alone and boosting with the complete vaccine candidate contributed towards an increase of the immune responses to the PD recombinant proteins as well as to total Leishmania antigens (TLA). This prime-boost immunization approach gives relevance to the use of both parasite and vector derived antigens together as an anti-Leishmania vaccine, and contributes to the debate on vaccination in endemic versus non-endemic areas, where people are either constantly or never (respectively) exposed to sand fly bites.

Acknowledgments:
This work was supported by MuLeVaClin (Clinical Studies on a Multivalent Vaccine for Human Visceral Leishmaniasis - project reference 603182) funded by FP7-HEALTH.

Correspondence: Email - pedro.cecilio@ibmc.up.pt

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Stigma: The hidden fiery spear of cutaneous Leishmaniasis in Ethiopia.

M Behailu [1], B Hilde [2], P V Jean [3], V G Johan [4*]

[1] Arba Minch University, Department of Public Health, College of Medicine and Health Sciences, Ethiopia; [2] Department of Primary and Interdisciplinary Care, Faculty of Medicine and Health Science, University of Antwerp-Belgium; [3] Faculty of Medicine and Health Sciences-Epidemiology and Biostatistics Unit Institute of global Health University of Antwerp, Belgium; [4] Institute of Tropical Medicine, Antwerp, Belgium.

Available Online: 30 December 2018

ABSTRACT

Background: Ochollo, a small village is an endemic focus for *Leishmania aethiopica* infection since its recognition of 1913.

The objective of the study was, to assess what psychological impacts the disease had put forth on people who have the active lesion or has the permanent scars the disease.

The Gap: At least until today, no studies done in Ethiopia to understand the psycho-social impact of the CL scar and/or ulcer.

The Hook: Being the first research to bring the issue of CL associated stigma on board the finding will help unveiling it for a better understanding of the psychological consequences it puts forth.

Results: Five main themes : Fear, Shame, Isolation, Stigma, and Rejection. Change in behavior started after individual contracted CL. Stigma: is the most common and repeatedly expressed form of psychosocial impact on people who are having or had CL. A father of a 12 year old 4th grade student from TK said, "When the lesion holds pus, then the discrimination occurs….." . At home when they sit for dining "…..give her own with another separate plate… or they say to her to cover her face ….In school and community same……. Another 48 years old male participant from KA saying, "My eyes were affected. One day while talking with a person he directly insulted me saying 'You man with the Bolbo'. After that, I was so ashamed, and psychologically traumatized and then I wanted even to remove the scar from my face by peeling off the scar from my face so that nobody insults me".

Conclusion and recommendation: Although, some CL lesions heal spontaneously leaving life-long scars, the majority of lesions are non-healing or chronic with sequels leading to disfigurement or mutilation with severe social and economic consequences. The results indicate that Cutaneous leishmaniasis put forth a psychological impact up on individuals who contracted the disease and having a lesion is attached to stigma.

Acknowledgments:
The Flemish universities cooperation for the financial support (Viliri-ous).

References:
Hotez PJ, Molyneux DH, Fenwick A, Kumaresan J, Sachs SE, et al., N Engl J Med. 357 (2007) 10 1018–1027. doi:10.1056/NEJMra064142.
Issam Bennis , Séverine Thys, Hind Filali, Vincent De Brouwere, Hamid Sahibi and Marleen Boelaert, Infectious Diseases of Poverty 6 (2017) 46. doi:10.1186/s40249-017-0267-5

Correspondence: Email - jvangriensven@itg.be

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Cloning, expression and production of rK39 from Iranian strain of Leishmania infantum for serodiagnosis of visceral leishmaniasis in human

Bibi Razieh Hosseini Farash [1, 2, 3], Mehdi Mohebali [3], Abdolmajid Fata [1,2], Seyed Aliakbar shamsian [2], Bahram Kazemi [4], Homa Hajjaran [3], Behnaz Akhoundi [3], Reza Raoofian [2,3].

[1] Cutaneous leishmaniasis Research Center, School of Medicine, Mashhad, Iran; [2] Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [3] School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [4] Faculty of Medicine, Shaheed Beheshti University of Medical Sciences, Tehran, Iran.

Available Online: 30 December 2018

ABSTRACT

Introduction: Visceral leishmaniasis(VL) caused by *Leishmania infantum* is the most severe form of leishmaniasis in Iran [1]. A high mortality rate has been reported without accurate diagnosis and treatment of VL [2]. This study aimed to investigate on gene cloning and preparation of K39 immunodominant antigen of Iranian strain of *L. infantum* and its evaluation on diagnosis of VL in Iran.

Material and Methods: The band of interest of k39 was ligated into pCR 2.1-TOPO and pET-32a (+), respectively after PCR amplification. The sequences of recombinant plasmids were analyzed. Protein expression and purification of rk39 were performed and along with, the whole cell and DAT antigens were prepared to compare the results with recombinant protein. For the final step, 84 positive and 86 negative serum samples of human for VL, are being collected to evaluate sensitivity and specificity of new recombinant antigen for serodiagnosis of VL.

Results: The sensitivity of rK39-ELISA has been reported 85.7% with specificity of 86% for diagnosis of Human VL (HVL) (≤1:800) while showed 100% sensitivity in symptomatic people (≤1:3200). The kappa index was calculated for this recombinant antigen to find the level of agreement with DAT. This value was 0.718 and has an excellent agreement with gold standard.

Conclusions: On the whole, the successful rk39 cloning and new recombinant antigen which have performed in this study could help to serodiagnosis of HVL especially in where *L. infantum* is the main causative agent. Furthermore, this would make this possibility to discuss about the real sensitivity of rK39 prepared by Iranian *L. infantum* and its application along rk26 protein to diagnosis VL in Iran.

Keywords: Visceral leishmaniasis, *Leishmania infantum*, K39 immunodominant antigen, human

References:

1] Mohebali M., J Parasitol. 8 (2013) 3 348–58.

2] Mohebali M, Edrissian GH, Shirzadi MR, Akhoundi B, Hajjaran H, Zarei Z, et al., Travel Med Infect Dis. 9 2011 2 67–74. doi:10.1016/j.tmaid.2011.02.003

Correspondence: Email - hoseinifr@mums.ac.ir

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

A double-sided perspective of leishmaniasis: the lab vs the field experiences

Carla Maia*, José Manuel Cristóvão, Sofia Cortes, Maria Odete Afonso, Ricardo Parreira, Lenea Campino

Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Lisboa, Portugal.

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis caused by the protozoan *Leishmania infantum* and transmitted by the phlebotomine sand flies Phlebotomus perniciosus and P. ariasi is an endemic zoonosis in Portugal [1]. Dogs are the major host for these parasites, and the main reservoir host for human infection.

The aim of this communication is to update the research on *Leishmania* infection in cats and vectors and *in vitro* drug susceptibility performed by Leishmaniases group of the Institute of Hygiene and Tropical Medicine, University Nova of Lisbon in the last years. *L. infantum* infection in cats has been evaluated in southern Portugal [2-4]. In addition, several phlebotominae surveys were carried out and vector species were found infected with L. infantum. DNA of *L. major* and *Leishmania* spp. has been detected in *Sergentomyia minuta* specimens [5-9]. Regarding in vitro studies, strains isolated from dogs showed low susceptibility to the drugs used in canine leishmaniasis therapy and artemisinin-derived trioxanes and nonclassic metallointercalators with dipyridophenazine seem to be good candidates for further studies in the context of leishmanial treatment [10-12]. Data reveal that Portugal is a hypoendemic country for human leishmaniases but the prevalence rate of canine and feline infections is a concern for the control of this zoonosis. The on-going and recurrent detection of leishmaniasis in dogs, cats, other mammals and vectors reinforces the importance of surveillance with systematic epidemiologic surveys. Increase awareness of the veterinary community, owners and public health authorities regarding this zoonosis is also warranted.

Acknowledgments:
C. Maia and S. Cortes have the support of the Portuguese Ministry of Education and Science (via Fundação para a Ciência e a Tecnologia, I.P.), through the Investigator Starting Grants IF/01302/2015 and IF/0773/2015, respectively.

References:

1] L. Campino, C. Maia, Acta Med Port 23 (2010) 859-864.
2] C. Maia, M. Nunes, L. Campino, Vector Borne Zoonotic Dis 8 (2008) 555-559. doi:10.1089/vbz.2007.0247
3] C. Maia, J. Gomes, J. Cristóvão, M. Nunes, A. Martins, E. Rebêlo, L. Campino, Vet Parasitol 174 (2010) 336-340. doi:10.1016/j.vetpar.2010.08.030
4] C. Maia, C. Ramos, M. Coimbra, F. Bastos, A. Martins, P. Pinto, M. Nunes, M. Vieira, L. Cardoso, L. Campino, Parasit Vectors 7 (2014) 115. doi:10.1186/1756-3305-7-115
5] C. Maia, M.O. Afonso, L. Neto, L. Dionísio, L. Campino , J Vector Borne Dis 4 (2009) 268-272.
6] C. Maia, L. Dionísio, M.O. Afonso, L. Neto, J. Cristóvão, L. Campino, Mem Inst Oswaldo Cruz 108 (2013) 481-487. doi:10.1590/S0074-02761080420130014
7] S. Branco, C. Alves-Pires, C. Maia, S. Cortes, J. Cristovão, L. Gonçalves, L. Campino, M.O. Afonso, Acta Trop 125 (2013) 339-348. doi:10.1016/j.actatropica.2012.12.008
8] L. Campino, S. Cortes, L. Dionísio, L. Neto, M.O. Afonso, C. Maia, Mem Inst Oswaldo Cruz 108 (2013) 516-518. doi:10.1590/S0074-02762013000400020
9] C. Maia, R. Parreira, J. Cristóvão, F. Freitas, M.O. Afonso, L. Campino, Parasit Vectors 8 (2015) 173. doi: 10.1186/s13071-015-0787-4
10] C. Maia C, M. Nunes, M. Marques, S. Henriques, N. Rolão, L. Campino, Exp Parasitol 135 (2013) 36-41. doi:10.1016/j.exppara.2013.05.015
11] S. Cortes, A. Albuquerque, L. Cabral, L. Lopes, L. Campino, M. Cristiano, Antimicrob Agents Chemother 59 (2015) 5032-5035. doi:10.1128/AAC.01305-09
12] J. Madureira, C. Ramos, M. Marques, C. Maia, B. de Sousa, L. Campino, M. Santana-Marques, N. Farrell, Inorg Chem 52 (2013) 8881-8894. doi:10.1021/ic401067d

Correspondence: Email - CarlaMaia@ihmt.unl.pt

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Arginine metabolism and tumor necrosis factor in leishmaniasis: basic research and clinical aspects

Christian Bogdan [1,2*]

[1] Institute of Clinical Microbiology, Immunology and Hygiene, Friedrich-Alexander-University (FAU) Erlangen-Nürnberg and University Hospital Erlangen; [2] Medical Immunology Campus Erlangen, FAU-Erlangen-Nürnberg

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis comprises a spectrum of vector-borne chronic cutaneous, mucocutaneous or visceral diseases that are caused by different species of the protozoan parasite *Leishmania*. Experimental mouse models have been extremely informative to study and understand the immunopathogenesis of leishmaniasis. Nitric oxide generated by inducible nitric oxide synthase (iNOS or NOS2) following activation of macrophages by interferon (IFN)-γ and tumor necrosis factor (TNF) was shown to be essential for the control of acute and latent cutaneous leishmaniasis elicited by *Leishmania (L.) major* due to direct anti-leishmanial and immunoregulatory effects. Recently, we found that TNF not only contributes to the induction of iNOS, but also suppresses the expression of the competing enzyme arginase (Arg) 1 *in vitro* and *in vivo* by an epigenetic mechanism, thereby helping to maintain the output of NO. In the lecture I will discuss whether Arg1 and/or Arg2 are required for (a) the healing of acute *L. major* infections, (b) the long-term persistence of *L. major* parasites, and (c) the progression of non-healing cutaneous leishmaniasis caused by *L. mexicana*. The clinical implications of this work will also be outlined

Correspondence: Email - christian.bogdan@uk-erlangen.de

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Prognosis in dogs with Leishmaniasis in the Netherlands

Christine Piek [1*], Marja de Jong [1], Aukje Rappoldt [1]

[1] Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

Available Online: 30 December 2018

ABSTRACT

In recent years the import of foreign stray dogs in the Netherlands has steadily increased from around 8,000 in 2012 to 11,310 dogs per year in 2015. This last number is with 7.5% a considerable part of the total yearly increase of dogs in the Netherlands in 2015. Leismaniasis is not an endemic disease in the Netherlands. As a direct consequence the Dutch veterinarian has to cope increasingly with the challenge of diagnosis and treatment of Leishmaniasis in dogs. The question is if the progression of clinical disease, response to treatment, and survival in these dogs is comparable to the course of the disease in geographic areas where Leishmania infections are endemic. As a preliminary answer to this question a retrospective study was set up with the aim to determine survival time, response to therapy, and the factors determining prognosis in a cohort of 47 dogs diagnosed with Leishmaniasis. The overall estimated Kaplan-Meier median survival time was 6.4 years (95% CI 1.8-11 years). Univariate Cox regression analysis identified protein-losing renal disease as a strong negative predictor of survival. Multivariate Cox regression analysis identified decrease in haematocrit, and increases in plasma creatinine and serum total protein concentrations as the major factors determining prognosis. Interestingly, survival in dogs classified based on the severity of clinical disease (Canine Leishmania Working Group) using the major criteria proteinuria and azotemia (IRIS stage 3-4 kidney disease, CLWG stage D) as distinguishing features was not significantly different [1,2].

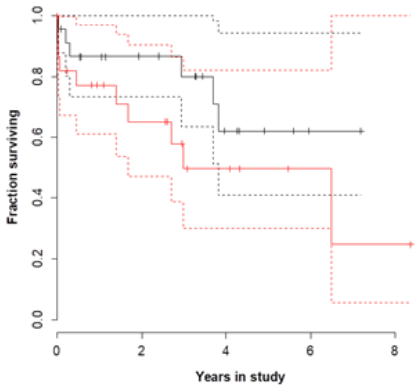

Figure 1 | Estimated survival in Kaplan-Meier survival curves for group A (red line) and B (black line). The dotted lines represent the corresponding 95% confidence intervals

References:
1] X. Roura, et al., The Veterinary Journal 198 (2013) 43–47. doi:10.1016/j.tvjl.2013.04.001
2] Solano-Gallego et al., Parasites & Vectors 4 (2011) 86. doi:10.1186/1756-3305-4-86

Correspondence: Email - C.J.Piek@uu.nl

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Clinical and laboratory features in human visceral leishmaniasis, northeastern Iran, 1997- 2017

Elham Moghaddas [1*], Seyed Aliakbar Shamsian [1], Abdolmajid Fata [1], Fatemeh Sadabadi [1], Mehdi Zarean [1], Zahra Navi [1]

[1] Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Available Online: 30 December 2018

ABSTRACT

Because of VL happens more in pediatrics at risk of death, accurate detection in an early stage of infection is necessary [1]. The aim of this study resolution of prevalence, clinical and laboratory findings visceral leishmaniasis patients compared to the healthy group. In this retrospective study, 25 confirmed hospitalized VL patients with 15 the healthy children were included between 1997- 2017 in Emam Reza hospital, Mashhad, Khorasan Province, Northeastern Iran. Gender, age, WBC, RBC, HGB, ESR, Hct, PT, PLT, AST, ALT, Na, K, BUN and RR were analyzed and compared to the healthy group by SPSS Ver. 20. During 20 years, twenty-five patients were recorded with a gender ratio of two males to one female. All cases presented with hepatosplenomegaly. Fever and RR in the patient group were statistically significant compared with the healthy group. Mean age of the patients was 3.7±4 years. No significant differences ($p>0.05$) were observed in ALT, K, PT between VL patients and control group. Na, HGB, PLT, and WBC were found the highly significant difference between case and control groups. The number of cases per year markedly decreased from 7.4 cases/year in the 1982- 1996, to 1.2 cases/year in the 1997- 2017. ALT, K, PT weren't reliable laboratory parameters in the diagnosis of VL in this study.

Acknowledgments:
We appreciated from Mashhad University of Medical sciences for financial support (Project grants: 960764).

References:
1] Miao R, Wang Z, Guo Q, Wen Y, Liao Q, Zhu Y, et al., The Pediatric infectious disease journal. 36 (2017) 1 9 –12. doi: 10.1097/INF.0000000000001343

Correspondence: Email - Moghaddase@mums.ac.ir

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Nature inspired new compounds against Leishmaniosis, from *Eremurus persicus*

Elisa Vignoni [1], Valeria Cavalloro [1], Serena Della Volpe [1], K M Ahmed [2], Marisa Machado [3], Maria Do Céu Sousa [4], Emanuela Martino [5], Simona Collina [1]*.

[1] Department of Drug Sciences, University of Pavia, Via Taramelli, 12, 27100 Pavia, Italy; [2] Department of Science-Chemistry, University of Garmian, Kalar, Kurdistan Region, Iraq; [3] CESPU, Instituto de Investigação e Formação Avançada em Ciências e Tecnologias da Saúde, 4585-116 Gandra PRD, Portugal; [4] Faculty of Pharmacy, University of Coimbra, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal; [5] Earth and Environmental Sciences Department, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy.

Available Online: 30 December 2018

ABSTRACT

To date, a limited number of drug candidates are available for fighting *Leishmania*, and there is an urgent need for efficient medical treatments. Within a wider research project aimed at discovering new bioactive compounds from plant kingdom, we focused on *Eremurus persicus* roots. As a result of our investigation, we isolated (*R*)-Aloesaponol III 8-methyl ether (*R*)-ASME, showing a remarkable antiprotozoal effect against *L. infantum* with an IC50 of 73 μg/mL and not significant toxicity in a macrophage cell line. The potential of such compound against *Leishmania* infections will lead us to get further insight into the mechanisms of actions studying its behavior in cells.[1]

Basing on the obtained results, we can state that (*R*)-ASME is a interesting *hit compound* and it constituted the starting point of the herein presented work. Of note, the hit is poorly soluble in water and in aqueous buffers, as a consequence the biological assays are difficult to perform and the results difficult to compare. Accordingly, we designed new water-soluble (*R*)-ASME-derivatives. To improve the *hit* physicochemical properties, different molecular modification strategies of (*R*)-ASME have been planned, taking into account its structural features and molecular reactivity. The investigated approaches consisted in the conjugation of (*R*)-ASME with either amino acid (AA), since it is considered a useful method for increase compound water solubility [2] or with an hydrophilic moiety. The latter approach allowed us to obtain a stable and water soluble salt. The *in vivo* assays of this ASME-derivative are still in progress.

Moreover, to improve the cellular uptake, and following a drug targeting approach [3],ongoing efforts will be addressed to prepare a biotin-conjugated (R)-ASME derivative. Results will be presented in due course.

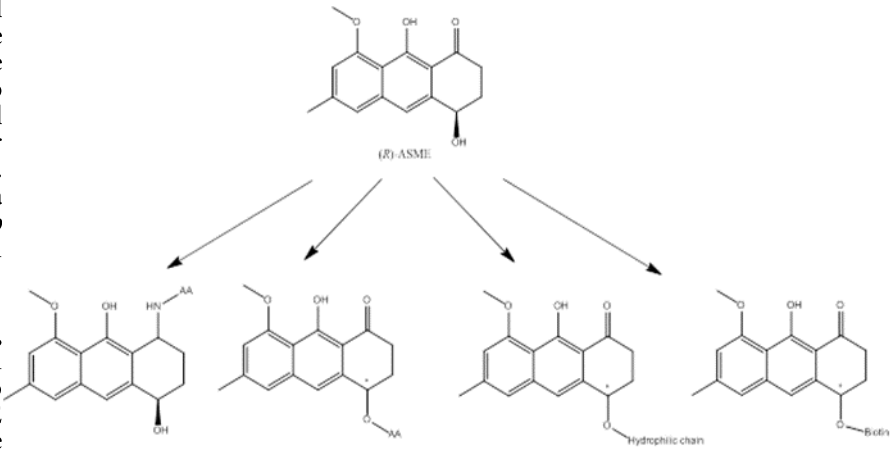

Figure 1 | Molecular modification strategies of (*R*)- ASME.

Correspondence: Email - simona.collina@unipv.it

References:

1] See oral communication. Martino E.,Cavalloro V., Vignoni E., Della Volpe S., Ahmed K.M. 3, Machado M., Do Céu Sousa M., Collina S. Nature aided drug discovery.Novel Compounds against Leishmaniosis from Eremurus persicus.

2] Mi Kyoung K. , Kwang-su P., Woon-seok Y., Hyunah C., Youhoon C., Bioorganic & Medicinal Chemistry, 17 (2009) 1164–1171. doi:10.1016/j.bmc.2008.12.043

3] Tripodo G., Mandracchia D., Collina S., Rui M.,Rossi D., Medicinal chemistry. (2014) 1-8. doi:10.4172/2161-0444.S1-004

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Leishmaniosis caused by *Leishmania tropica* and *Leishmania major* in dogs

Gad Baneth [1*], Yaarit Nachum-Biala [1]

[1] Koret School of Veterinary Medicine, The Hebrew University, P.O. Box 12, Rehovot 7610001, Israel.

Available Online: 30 December 2018

ABSTRACT

The main cause of canine leishmaniosis worldwide is *Leishmania infantum*. Clinical disease and infection associated with *Leishmania tropica* and *Leishmania major*, two common agents of human cutaneous leishmaniasis in the Middle East and North Africa, have rarely been reported in dogs. This study reports cases of canine infection with *L. tropica* and *L. major* with clinical manifestations in Israel and compares them to dogs with these infections reported from other countries and canine *L. infantum* infection. The identity of the infecting agent was determined by PCR and sequencing. Comparative serology by ELISA was performed using crude promastigote antigen of *L. tropica*, *L. major* and *L. infantum*. Skin lesions in *L. major* infected dogs were ulcerative and located on the muzzle, feet and foot pads and not associated with generalized lymphadenomegaly and splenomegaly. In *L. tropica* infection, skin lesions were proliferative mucocutaneous in young dogs, or associated with widespread dermatitis, lymphadenomegaly and splenomegaly in older dogs with similarity to *L. infantum* infection. ELISA serology with the different whole promastigote antigens seperately was not distinctive between *L. infantum*, *L. major* and *L. tropica* canine infections. In summary, *L. tropica* and *L. major* cause clinical disease in dogs. Dogs suspected of leishmaniosis in areas endemic for human infection with these species and with *L. infantum* should be tested by PCR with DNA sequencing to detect infection and discriminate between the three species. The possible role of canines as reservoirs for *Leishmania* species other than *L. infantum* should be further studied.

Correspondence: Email - gad.baneth@mail.huji.ac.il

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Conventional antileishmanial drug associated with membrane transport modulators represents a new strategy to leishmaniasis treatment

M. Rafael-Fernandes [1], A. Armada [1], G. Alexandre-Pires [2], A. Valério-Bolas [1], A. Rodrigues [1], M. Viveiros [1], G. Santos-Gomes [1]

[1] Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisboa, Portugal; [2] CIISA, Centre for Interdisciplinary Research in Animal Health. Faculty of Veterinary Medicine, University of Lisbon, Lisbon, Portugal

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis is among the parasitic diseases with the greatest impact in the world. It is considered a neglected disease that affects mainly the poorest populations with high morbidity. Furthermore, the few conventional drugs available for leishmaniasis treatment have high toxicity and limited efficacy. Thus, the development of new drugs or new treatment strategies is urgently needed. The efflux pump activity of ABC (ATP-binding cassette) transporters present on *Leishmania*-host cell membrane and on parasite may influence the internalization and permanence of drugs within the cells, conditioning the therapeutic activity and favoring parasite survival. Therefore, the present study aims to analyze the efficacy of miltefosine in combination with efflux pumps modulators (EPMs) in reducing intracellular infection. Mouse macrophages infected with *Leishmania* parasites causing zoonotic visceral leishmaniasis and American cutaneous leishmaniasis were treated with miltefosine in association with EPMs and analyzed for parasite viability. Although EPMs effect differs between *Leishmania* spp., the association of verapamil (VER), sodium orthovanadate and phe-arg β-naphthylamide (PAβN) with miltefosine seems to restrain drug efflux. VER, which modulates ABCB1 pump efflux and PAβN that modifies the activity of AcrAB transporters establish a synergic relation with miltefosine. The use of EPMs in a combined therapy should be strongly considered, since they may potentiate drug activity, reducing the drug concentration required to inactivate the parasite and, consequently diminish toxic effects and prevent the appearance of resistance. EPM-antileishmanial drug combined treatment can constitute an alternative therapeutic strategy to leishmaniasis conventional treatment.

Acknowledgments:
Portuguese Foundation for Science and Technology through GHTM – UID/Multi/04413/2013.

Correspondence: Email - gpires@fmv.utl.pt

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Molecular mass-screening for vector research on leishmaniasis

Hirotomo Kato [1*], Abraham G. Cáceres [2], Eduardo A. Gomez [3], Yoshihisa Hashiguchi [3]

[1] Division of Medical Zoology, Jichi Medical University, Japan; [2] Laboratorio de Entomología, Instituto Nacional de Salud, Peru; [3] Departamento de Parasitologia y Medicina Tropical, Facultad de Ciencias Medicas, Universidad Catolica de Santiago de Guayaquil, Ecuador.

Available Online: 30 December 2018

ABSTRACT

Surveillance of circulating sand fly species in endemic and surrounding areas and identification of sand flies responsible for transmission of *Leishmania* is important for predicting the risk and expansion of the disease. A molecular mass screening that can detect natural infection of *Leishmania* in hundreds of sand flies with minimum effort was established (Fig.1) [1]. Sand fly species were simultaneously identified by PCR-RFLP of the18S rRNA gene using same DNA extract as a template (Fig.2) [2,3]. This method was applied to field research in Ecuadorian and Peruvian Andes, and 3 of 192 and 1 of 462 sand flies, respectively, were positive for *Leishmania* minicircle DNA. In these areas, Lutzomyia ayacuchensis and Lu. peruensis were identified as vectors of *Leishmania* (*Leishmania) mexicana* in Ecuador and *L. (Viannia) peruviana* in Peru, respectively [4], corresponding to previous reports. Further, the natural infection of sand flies by *Leishmania* was examined in the Department of Huanuco of Peru, in which cutaneous leishmaniasis caused by a hybrid of L*. (V.) braziliensis/L. (V.) peruviana* is endemic and the vector is not known. A total of 2,997 female sand flies were analyzed, and a hybrid of *L. (V.) braziliensis/L. (V.) peruviana* was detected from one *Lu. tejadai*, strongly suggesting that *Lu. tejadai* is responsible for the transmission of the hybrid *Leishmania* circulating in this area [5]. In addition, loop-mediated isothermal amplification (LAMP), which can amplify target gene within 1 hour under isothermal conditions, was adopted as an alternative to PCR for mass-screening of sand flies [6]. Since these methods can process a large number of samples with minimum effort, they will be powerful tools for vector research on leishmaniasis.

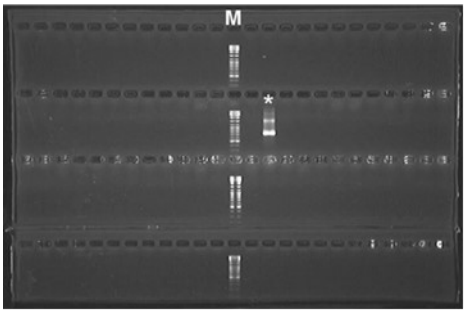

Figure 1 | Detection of *Leishmania* DNA within sand flies

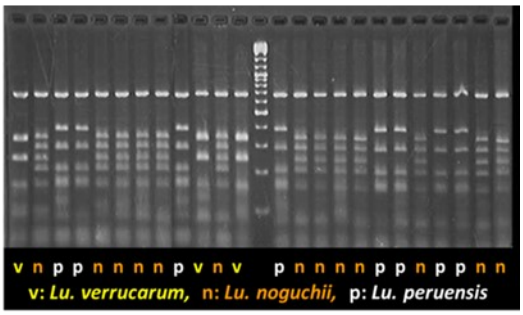

Figure 2 | Genotyping of sand flies

Correspondence: Email - hirok@jichi.ac.jp

References:

1] H. Kato, H. Uezato, E.A. Gomez, Y. Terayama, M. Calvopiña, H. Iwata, Y. Hashiguchi, Am J Trop Med Hyg. 77 (2007) 324-329. doi:10.4269/ajtmh.2007.77.324

2] Y. Terayama, H. Kato, E.A. Gomez, H. Uezato, M. Calvopiña, H. Iwata, Y. Hashiguchi, J Vet Med Sci. 70 (2008) 907-913.

3] M. Fujita, H. Kato, A.G. Cáceres, E.A. Gomez, L. Velez, T. Mimori, F. Zhang, H. Iwata, M. Korenaga, T. Sakurai, K. Katakura, Y. Hashiguchi, Acta Trop. 121 (2012) 93-98. doi:10.1016/j.actatropica.2011.10.004

4] H. Kato, A.G. Caceres, E.A. Gomez, T. Mimori, H. Uezato, J.D. Marco, P.A. Barroso, H. Iwata, Y. Hashiguchi, Am J Trop Med Hyg. 79 (2008) 719-721. doi:10.4269/ajtmh.2008.79.719

5] H. Kato, A.G. Cáceres, Y. Hashiguchi, PLoS Negl Trop Dis. 10 (2016). doi:10.1371/journal.pntd.0004336

6] C.O. Nzelu, E.A. Gomez, A.G. Cáceres, T. Sakurai, L. Martini-Robles, H. Uezato, T. Mimori, K. Katakura, Y. Hashiguchi, H. Kato, Acta Trop. 132 (2014) 1-6. doi:10.1016/j.actatropica.2013.12.016

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

NOD2 pathway implication in *Leishmania tropica* infection

I Varotto-Boccazzi [1], D Scaccabarozzi [2], S Villani [3], S Zava [2], L Cavicchini [3], R Nodari [1], S Delbue [3], I Colombo [2], S Epis [1], N Basilico [3], Y Corbett [1,2]

[1] Department of Biosciences and Pediatric Clinical Research Center, University of Milan, Italy; [2] Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy; [3] Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy.

Available Online: 30 December 2018

ABSTRACT

Leishmania tropica is the etiological agent of cutaneous leishmaniasis. Pattern recognition receptors such as toll-like receptors or nucleotide oligomerization domain-like receptors (NLR) have been associated with the disease. The role of NOD2, a member of the NLR family, in innate immune responses against *L. tropica* has been investigated.

Interferon gamma (IFN-γ)-primed or unprimed immortalized mouse bone marrow macrophages (BMDM)-wild type (WT) or -NOD2$^{-/-}$ were infected with L. tropica at different parasite/macrophage ratios (i.e. 2.5, 5, or 10:1), for 24h. Controls, such as medium alone, muramyldipeptide (MDP), or lipopolysaccharide (LPS), were included. Levels of cytokines or nitrite released into supernatants were measured through ELISA or Griess reagents. Levels of inducible nitric oxide synthase (iNOS) mRNA and protein were obtained through Real-Time PCR and Western blot analyses, respectively. Also, unprimed BMDM-RIP2$^{-/-}$ or -CARD9$^{-/-}$, two downstream components of NOD2 activation, were stimulated with the same ratios of *L. tropica* or controls, and the production of the pro-inflammatory cytokine TNF-a was compared with BMDM-WT.

Data showed that *L. tropica* did not induce the production of nitric oxide in unprimed BMDM-WT. *L. tropica* induced higher levels of nitric oxide in IFN-γ-primed BMDM-WT than in unstimulated cells. Stimulation due to *L. tropica*, or the control MDP (known to activate NOD2), was abrogated in the BMDM-NOD2$^{-/-}$, but not LPS, as so the expression levels of inducible nitric oxide synthase (iNOS) mRNA or protein. In addition, NOD2, RIP2 or CARD9 showed to be involved in the induction of TNF-a release from BMDM. These data suggest an involvement of NOD2 pathway in innate immune response to *L. tropica* infection.

Correspondence: Email - ilaria.varotto@unimi.it

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

A new epitope-based peptide vaccine against human leishmaniasis

Joana Pissarra [1]*, Philippe Holzmuller [2], Rachel Bras-Gonçalves [1], Amel Garnaoui [3], Jean-Loup Lemesre [1].

[1] UMR 177 INTERTRYP IRD, CIRAD, Univ. Montpellier (I-MUSE), Montpellier, France; [2] UMR CMAEE, Centre de coopération International en Recherche Agronomique pour le Développement (CIRAD), Montpellier, France; [3] Laboratoire d'Immunologie, Vaccinologie et Génétique Moléculaire, Institut Pasteur de Tunis (IPT), Tunis, Tunisia.

Available Online: 30 December 2018

ABSTRACT

Vaccine development against leishmaniasis is supported by evidence of natural immunity against infection, mediated by a dominant Th1 response and production of IFN-γ, IL-2 and TNF-α by polyfunctional CD4+ and CD8+ T cells, ultimately leading to macrophage activation and parasite killing. The discovery of immunodominant epitopes responsible for natural protection remains a challenge and a hurdle to vaccine development.

Excreted-secreted proteins are important virulence factors present throughout Leishmania life stages and induce durable protection against infection in dogs. Our rationale for the development of a human vaccine is to identify the immunodominant epitopes present in the Leishmania secretome, and design and synthesize peptides able to induce robust and durable immunity against all major parasitic stages and across the most clinically relevant species of *Leishmania*.

The secretome of 6 main pathogenic species was identified by Mass Spectrometry and conserved candidate antigens were searched. We selected a total of 52 protein candidates – corresponding to 28 proteins previously described as vaccine antigen candidates, and 24 new antigen candidates through a reverse vaccinology approach. The selected proteins underwent in silico HLA-I and –II epitope binding prediction analysis, with world coverage regarding HLA restriction. Strong binders were selected through an automated R script developed in-house, according to strict criteria. The automated script allowed us to greatly restrict the epitope list, and ultimately select 50 HLA-I and 24 HLA-II epitopes, synthesized as peptides.

Experimental validation of selected peptides is ongoing, wherein we aim to demonstrate their ability to induce Th1 and cytotoxic responses in human cells, from different immunity status (healed, asymptomatic and naïve). To test pre-existent *Leishmania*-specific memory responses, total PBMC assays are performed to detect IFN-γ production and other-Th1 associated markers. To test the naïve repertoire for specific precursors, T-cell co-culture assays are performed, with several rounds of cellular amplification due to the rarity of specific cells, and search for specific IFN-γ production by ELISpot. Promising peptide candidates will proceed to multi-epitope peptide design, which will be further tested and included in a final vaccine formulation.

Through the combination of proteomic analysis and in silico tools we were able to swiftly identify promising antigen candidates. We further established the secretome as an optimal starting point for vaccine development and the proposed in silico pipeline provides a rapid selection of the best epitopes, with great immunogenic potential. Validation of candidate peptides is performed exclusively with human samples, enabling conclusive immunogenicity testing while avoiding high-cost animal trials with limited extrapolation for human immunity. Together, the proposed strategy will provide us a very strong base for a vaccine formulation against human leishmaniasis and allow to fast-track translation to the field.

Acknowledgments:
This project has received funding from the European Union's horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 642609.

Correspondence: Email - joana.pissarra@ird.fr

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Leishmanicidal activity of 7-amino-1,2,4-triazolo[1,5-a]pyrimidine Cu(II) complexes

J M Méndez-Arriaga [1,2*], I Oyarzabal [3], G Escolano [4], A Rodríguez-Diéguez [1], M Sánchez-Moreno [2], J M Salas [1]

[1] Universidad de Granada, Dpto. Química Inorgánica, Avda. Fuentenueva, 18071 Granada, Spain; [2] Universidad de Granada, Dpto. Parasitología, Avda. Fuentenueva, 18071 Granada, Spain; [3] Université de Bordeaux, CNRS, CRPP, UPR 8641, 33600 Pessac, France; 4 Universidad de Granada, Dpto. Química Orgánica, Avda. Fuentenueva, 18071 Granada, Spain.

Available Online: 30 December 2018

ABSTRACT

Looking for the design of new drug candidates for potent, selective and less toxic leishmanicidal therapeutic agents, our research group has been working with triazolopyrimidine derivatives to form new antiparasitic metal complexes during last decades [1]. Two triazolopyrimidine complexes have been obtained from reaction between 7-amino-1,2,4-triazolo[1,5-a]pyrimidine (7atp) and Cu (II) salts. Crystal structures of $[Cu_2(\mu\text{-}7atp)4Cl_2]Cl_2{\cdot}4H_2O$ (1) and $[Cu_2(\mu\text{-}7atp)4(H_2O)_2](NO_3)_4{\cdot}H_2O$ (2) have been studied by X-ray diffraction methods and characterized by spectroscopic and thermal analysis. Magnetic studies of these dinuclear complexes have revealed the existence of moderate antiferromagnetic interactions between the copper ions, with J values of -91.2 and -96.1 cm^{-1} respectively. The antiparasitic activity of these new complexes has been studied in vitro against three different strains of Leishmania spp. and Trypanosoma cruzi, showing a higher efficacy than the 7atp ligand and the reference commercial drugs Glucantime and Benznidazole [2].

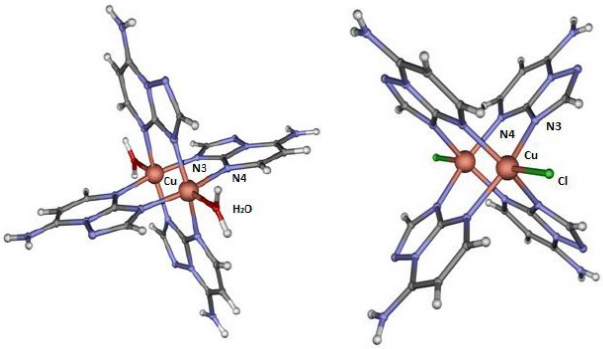

Figure 1 | Perspective view of complexes 1-2

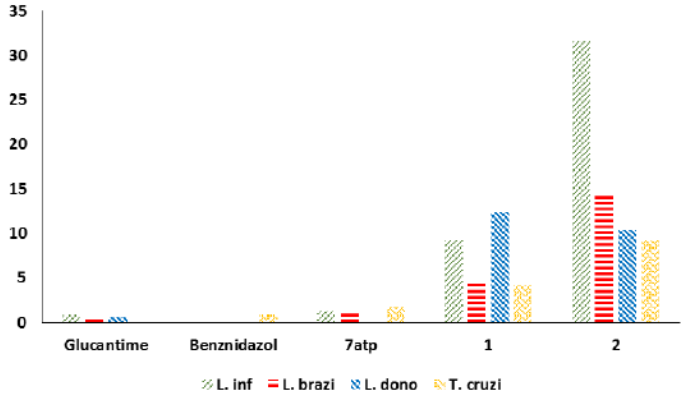

Figure 1 | Comparative SI values between reference drugs, pure 7atp ligand and complexes.

Acknowledgments:
This work was carried out in the frame of the "Red de iones metálicos en sistemas biológicos. Red de Excelencia CTQ2015-71211-REDT" network and the Junta de Andalucía (FQM-1484 and FQM-195).

References:

[1] J.M. Salas, M.A. Romero, M.P. Sánchez, M. Quirós, Coord. Chem. Rev 193-195 (1999) 1119–1142
[2] J.M. Méndez-Arriaga, I. Oyarzabal, G. Escolano, A. Rodríguez-Diéguez, M. Sánchez-Moreno, J.M. Salas, J. Inorg. Biochem. 180 (2018) 26 -32. doi:10.1016/j.jinorgbio.2017.11.027

Correspondence: Email - qmendez@ugr.es

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Characterization of a new focus of visceral leishmaniasis: canine, human and vector components

Joziana Muniz de Paiva Barçante [1*], Sidney Ferreira [1], Thiago Pasqua Narciso [2], Richardson Costa Carvalho [1], Ingrid Marciano Alvarenga [2], Alexandra Almeida Pinheiro Chagas [1], Marina Martins Oliveira [2], Thales Augusto Barçante [1]

[1] Health Science Department, Federal University of Lavras; [2] Veterinary Medicine Department, Federal University of Lavras

Available Online: 30 December 2018

ABSTRACT

Visceral leishmaniasis (VL), or Kalazar, is a vector-borne disease that continues to increase although the classical control measures have been implemented. VL presents high lethality in the cases not treated properly and has spread around the world. This neglected disease is underestimated since it is unnotified in many endemic countries. With the emergence of foci of the disease in urban areas, VL represents a significant burden in public health in Brazil, which is among the six countries that harbor over 90% of the cases worldwide [1,2]. All regions of this country are related to the presence of VL. In the State of Minas Gerais (MG), the disease has spread dramatically in the last 59 years. In this scenario, human and canine cases and infected vectors have been registered to suggest a clear expansion of disease. Therefore, it is essential that health professionals be familiar with the clinical patterns of VL to proceed with the correct diagnosis and proper treatment soon [3,4]. Considering that canine cases usually preceds human VL [5-7], the early detection of new geographical areas with canine VL cases is a critical point for starting or improving the epidemiological surveillance of leishmaniasis. Since the first confirmed case of canine VL occurred in 2013 and the first report of Lutzomyia longipalpis, in 2015 in the municipality of Lavras, health education actions have been implemented by the authors. In January 2017, the first autochthonous case of human VL was diagnosed, which elevated to 226 the number of municipalities with reported cases of human VL in the State of MG. As health education is considered an essential resource to control leishmaniasis7, the educational actions have been applied to the health professionals, teachers of elementary education and high schools and the broad community by the distribution of informative brochures, flipcharts, and interactive lectures. After this first report of human VL other five cases were diagnosed in the city of Lavras in 2017 including adults, children, and an elderly man. These reports highlight the need of maintenance of surveillance and control programs including the active search of sandflies, human and canine cases and also public health education. The current situation of Lavras should also be considered as an alert to other near areas where favorable eco-epidemiological conditions of transmission may exist. We believe that the answer to VL challenges is health education and community participation – two activities that do not appear to be high priorities in the majority of endemic areas. The understanding and active participation of the community is pivotal to get success in the control of the disease. With long-term political support, adequate funding and continuous educational programs, we can be able to control VL and virtually any neglected disease. In summary, health education is a precious key to solve our social problems.

References:

1] Sousa-Gomes M L, Romero G A, Werneck G L., Plos Negl Tropic Dis 11 (2017). doi:10.1371/journal.pntd.0005772.

2] Marzochi M C A, Coutinho S G, Souza W, Toledo L M, Grimaldi Jr G, Momen H, Pacheco R S, et al., Mem Inst Oswaldo Cruz. 80 (1985) 349-357. doi:10.1590/S0074-02761985000300012

3] Brasil, Ministério da Saúde. Manual de Vigilância e Controle da Leishmaniose Tegumentar Americana. Secretaria de Vigilância em Saúde, Departamento de Vigilância Epidemiológica. 2017; Brasília/DF.

4] Silva GA, Boechat TO, Ferry FR, Pinto JF, Azevedo MC, Carvalho RS, et al, Rev Inst Med Trop Sao Paulo 56 2014 81-84. doi:10.1590/S0036-46652014000100013

5] Werneck G L, Cad Saude Publica 24 (2008) 2937-2940. doi:10.1590/S0102-311X2008001200023

6] Quinnell R J, Courtenay O, Parasitology 136 (2009) 1915-1934. doi:10.1017/S0031182009991156

7] Brasil. Ministério da Saúde. Casos confirmados de Leishmaniose Visceral, Brasil, Grandes Regiões e Unidades Federadas. 1990 a 2010. Secretaria de Vigilância em Saúde. Brasília: Ministério da Saúde; 2011.

Correspondence: Email - joziana@dsa.ufla.br

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Evolutionary relationships among protein deacetylases of *Leishmania* and other parasites

Laila Alves Nahum [1,2*], Larissa Lopes Silva Scholte [3]

[1] René Rachou Institute, Oswaldo Cruz Foundation - FIOCRUZ, MG 30190-009, Brazil; [2] Promove College of Technology, Belo Horizonte, MG 30140-061, Brazil; [3] Oswaldo Cruz Foundation - FIOCRUZ, RJ 21040-361, Brazil.

Available Online: 30 December 2018

ABSTRACT

Histone modifying enzymes are potential drug candidates against neglected diseases. They are involved in the regulation of chromatin modifications, thus globally regulating gene expression. Moreover, aberrant epigenetic states are often associated with human diseases, leading to great interest in these enzymes as therapeutic targets. We have analyzed two families of protein lysine deacetylases (HDACs and sirtuins) of humans, *Leishmania (L. braziliensis, L. donovani, L. major, and L. mexicana), Trypanosoma (T. brucei and T. cruzi), Schistosoma (S. japonicum, S. haematobium, and S. mansoni),* and *Plasmodium falciparum.* Potential homologues in the predicted proteomes of these taxa were identified by using hidden Markov model profiles. We reconstructed the evolutionary relationships of protein sequences by Bayesian inference and maximum likelihood method. Our results showed that parasite proteomes have diverse protein deacetylases (HDACs and sirtuins) and the evolutionary relationships among them are well supported. Experimental data described elsewhere suggests these enzymes as common drug targets among parasites. Our work has improved the functional annotation of approximately 63% HDACs and 51% sirtuins in the selected taxa providing insights for future experimental characterization. Together, our work contributes to a better understanding of parasite proteins and might support the development of new inhibitors and strategies against human diseases.

Acknowledgments:
CAPES (PICT-2012-3044), Centre National de la Recherche Scientifique (CNRS) (UMR 8204), CNPq (470673/2014-1, 304138/2014-2, 168223/2014-7, and 307479/2016-1), European Commission Seventh Framework Programme (A-ParaDDisE, 602080), European Regional Development Fund of the European Commission, FAPEMIG (PPM-00189-13 and RED-00014-1), and Vale Technological Institute/ FIOCRUZ (22298). We thank our collaborators in the article aforementioned. We also thank Francislon Oliveira (FIOCRUZ) for the Perl scripts used in our work.

References:
1] L.L.S. Scholte, M.M. Mourão, F.S. Pais, J. Melesina, D. Robaa, A.C. Volpini, W. Sippl W, R.J. Pierce, G. Oliveira, L.A., Nahum. Infect Genet Evol. 53 (2017) 175-188. doi:10.1016/j.meegid.2017.05.011

Correspondence: Email - laila@minas.fiocruz.br

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Levels of trace elements in sérum of dogs and their correlation to occurrence of leishmaniasis

Raffaele Corso [1*], Mauro Esposito [2], Giuseppe Picazio [2], Pasquale Gallo [2], Laura Manna [1]

[1] Dipartimento di Medicina Veterinaria e Produzioni Animali, Università degli Studi di Napoli; [2] Istituto Zooprofilattico Sperimentale del Mezzogiorno, Portici.

Available Online: 30 December 2018

ABSTRACT

The chemical contamination of the environment is a considerably serious problem. The majority of dangerous chemical pollutants, considered particularly harmful for humans, especially children [1], are heavy metals, relating to water and soil contamination [1-3]. The main threats to human health are associated with exposure to lead, cadmium, mercury and arsenic. Although adverse health effects of heavy metals have been known for a long time, exposure to heavy metals continues and is even increasing in some areas [2]. The World Health Organization (WHO) estimates that about a quarter of all diseases are due to prolonged exposure to environmental pollution. Certain heavy metals have been reported to seriously affect the immune system potentially resulting in a broad range of harmful health effects. The link between metals and immune function has been studied for many years; developmental exposure to lead results in persistent immune alterations in rodents, including reduced antibody levels, altered cytokine production [4]. Some studies were conducted on the heavy metal content in serum of dogs to evaluate the degree of exposure in urban or industrial areas [5]. Another study checked a potential link among histopathology and some trace elements in canine visceral leishmaniasis, a severe and fatal systemic chronic inflammatory disease [6]. The present study was aimed at determining trace element concentration in serum of dogs to evaluated if high levels of heavy metals are a factor contributing to vulnerability to leishmaniasis. Blood samples were collected from 19 leishmaniotic dogs and 74 not leishmaniotic dogs. All the 93 animals were from different geographic areas of Campania Region, endemic for *L. infantum*.

The analysis was carried out using a validated analytical method based on inductively coupled plasma massspectrometry (ICP-MS), and the data recorded were statistically processed in order to give a contribution to risk assessment. Blood samples obtained from dogs were kept at room temperature for 30 min and centrifuged at 3000 rpm for 15 min to separate the serum. The serum samples were transferred in eppendorf tubes and stored at −80°C until analysis. Aliquots (500 µL) of serum samples were transferred in metal- free polyethylene tube and they were diluted to 10.0 mL with HNO_3 1% (v/v).

The determination of 16 trace elements (As, Hg, Pb, Cd, As, Sr, V, Ni, Se, Cr, Mo, Li, Cu, Zn Mn and Fe) was carried out by an ICP-MS mod NexION 350X (Perkin Elmer, Waltham, MA-USA). All measurements were conducted in duplicate. Trace element concentrations were calculated by using calibration curves and were expressed as mg/L. The limits of quantification (LOQ) were calculated as the blank signal plus ten times its standard deviation, respectively. Monitoring of trace elements was carried out. Regarding the differences between not leishmaniotic versus leishmaniotic dogs, the results obteined in this preliminary study showed that: the mean quantity of Fe was 4,259 µg/mL ± sd vs 2,763 µg/mL ± sd, Mn was 0,006 µg/mL ± sd vs 0,008 µg/mL ± sd, Sr was 0,057 µg/mL vs 0,064 µg/mL. The differences between the average quantities of the other metals did not show statistically significant differences. Future studies will be needed to assess the correlation of leishmaniosis with serum metal concentrations.

References:
1] Velea T et al., Environmental Science and Pollution Research, 16:24-32, 2009.
2] Järup L. British Medical Bulletin, 68:167-182, 2003.
3] Zhuang P et al. Journal of Environmental Sciences, 21:849-53, 2008.
4] Dietert RR et al. Toxicol Appl Pharmacol. 198:86-94, 2004.
5] Tomza-Marciniak A et al. Biol Trace Elem Res. 149:345-51,2012.
6]Souza CC et al. Int J Exp Pathol. 95(4):260-70, 2014

Correspondence: Email - raffaelecorso@gmail.com

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

An overview on Leishmaniases in Portugal: epidemiology, clinical presentations and therapy

Lenea Campino [1]

[1] Instituto de Higiene e Medicina Tropical/ Global Health and Tropical Medicine, Universidade NOVA de Lisboa, Portugal.

Available Online: 30 December 2018

ABSTRACT

In Portugal, human visceral leishmaniasis (HVL) is hypoendemic like in other Western European countries. HVL had a high resurgence in Europe in the 1980s and 1990s due to the emergence of the pandemic HIV infection. In recent years, the incidence of co-infection cases has declined sharply. All the notified co-infection cases were in adult patients. Regarding the clinical cutaneous form (CL), only a few cases are presently diagnosed, due to the benign form of the infection, more often being self-healing. More severe cases of CL are found in patients with immunosuppression such as diabetes.

In opposition, canine leishmaniasis (CanL) prevalence continues to reach high infection rates. Last national serological survey has shown prevalence higher to 15%, in some regions of the interior Centre and South of the country.

In Portugal, treatment of HVL with liposomal amphotericin B has a high success rate being the first line treatment. Concerning CanL, the treatment is very diverse and the results are weak.

Correspondence: Email - campino@ihmt.unl.pt

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Guanylate binding proteins 2b and 5 as indicators of inflammation during leishmaniasis

Marie Lipoldová [1*], Yahya Sohrabi [1], Valeriya Volkova [1], Tatyana Kobets [1], Helena Havelková [1], Imtissal Krayem [1], Martina Slapničková [1], Peter Demant [2].

[1] Institute of Molecular Genetics of the Czech Academy of Sciences, Prague, Czech Republic; [2] Roswell Park Cancer Institute, Buffalo, USA

Available Online: 30 December 2018

ABSTRACT

Interferon-induced GTPases (guanylate-binding proteins, GBPs) play an important role in inflammasome activation and mediate innate resistance to many intracellular pathogens [1], but little is known about their role in leishmaniasis. We therefore studied expression of *Gbp2b/Gbp1* and *Gbp5* mRNA in skin, inguinal lymph nodes, spleen and liver after *Leishmania major* infection and in uninfected controls. We used two different groups of related mouse strains: BALB/c, STS and CcS-5, CcS-16 and CcS-20 that carry different combinations of BALB/c and STS genomes, and strains O20, C57BL/10 (B10) and B10.O20, OcB -9 and OcB-43 carrying different combinations of O20 and B10 genomes. The strains were classified on basis of size and number of infection-induced skin lesions as highly susceptible (BALB/c, CcS-16), susceptible (B10.O20), intermediate (CcS-20), and resistant (STS, O20, B10, OcB-9, OcB-43). All tested strains harbored parasites after infection, although the parasite load in resistant strains is low. Some uninfected strains differed in expression of *Gbp2b/Gbp1* and *Gbp5*, especially of *Gbp2b/Gbp1* in skin. Uninfected BALB/c and STS did not differ in their expression, but in CcS-5, CcS-16, and CcS-20, which all carry BALB/c-derived Gbp gene-cluster, expression of *Gbp2b/Gbp1* exceeds that of both parents. These data indicate trans-regulation of Gbps - pattern of inheritance which is considered to be caused by trans-regulatory effects of non-linked or distant genes. Infection resulted in approximately 10x upregulation of *Gbp2b/Gbp1* and Gbp5 mRNAs in organs of both susceptible and resistant strains, which was most pronounced in skin. CcS-20 expressed higher level of *Gbp2b/Gbp1* than both parental strains in skin, whereas CcS-16 expressed higher level of *Gbp2b/Gbp1* than both parental strains in skin in liver. This indicates a trans-regulation present in infected mice CcS-16 and CcS-20. Immunostaining of skin of five strains revealed in resistant and intermediate strains STS, CcS-5, O20 and CcS-20 tight co-localization of *GBP2b/GBP1* protein with most L. major parasites, whereas in the highly susceptible strain BALB/c most parasites did not associate with *GBP2b/GBP1*.

Conclusion: Expression of Gbp2b/Gbp1 and Gbp5 was increased even in organs of clinically asymptomatic resistant mice. It suggests a hidden inflammation, which might contribute to control of persisting parasites. This is supported by the co-localization of GBP2b/GBP1 protein and L. major parasites in skin of resistant and intermediate but not highly susceptible mice [2].

Acknowledgments:
This work was funded by the Czech Science Foundation (Grants GACR 14-30186S, GACR 16-22346S).

References:
1] E. Meunier, P. Broz, Cell Microbiol 18 (2016) 168–180. doi:10.1111/cmi.12546
2] Y. Sohrabi, V. Volkova, T. Kobets, H. Havelková H, I. Krayem et al., Frontiers Immunol 9 (2018) 130. doi:10.3389/fimmu.2018.00130

Correspondence: Email - lipoldova@img.cas.cz

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Visceral Leishmaniasis Discovered in Northern Somalia

Mikko Aalto [1]*.

[1] Bosaso General Hospital, Bosaso, Somalia;

Available Online: 30 December 2018

ABSTRACT

Discovering a new disease like Visceral Leishmaniasis (VL) in a geographic area where it is both unknown and unknown to exist is difficult if diagnosing needs special tests which are not available, just because the disease is not known to exist. In the Northern Somalia there had been long time small children with waisting, pancytopenia and splenomegaly and ending always fatally. As VL had been unknown to local doctors, these children were ”diagnosed” with leukemia, for which no treatment was available any case.

Because no rK39 RDTs were not available, first few suspicions in 2014 were checked by bone marrow biopsy and making Giemsa stained imprints from them. Without special expertise the microscopy for amastigotes proved out to be very difficult and several hours of microscopy was needed to be moderately convinced that ”the observed objects” are really amastigotes. On contrary the dramatic and rapid cure with Sodium Stibogluconate (SSG) proved much convincingly the diagnosis – or at least that a good treatment was found. Only after and because of a few children being diagnosed with VL and treated successfully we received from WHO also rK39 RDTs (Kalazar Detect™ InBios International Inc.). However these RDTs seemed to have sensitivity of around 70% and were negative with many children who had very advanced state of VL and who responded well and were cured with SSG. Hence in 2016 all suspected patients had combination of RDT, bone marrow or spleen aspirate Giemsa staining for amastigotes and promastigote culture done. Also Malaria RDTs, HIV tests, chest x-rays, blood cultures etc were used routinely for other pathologies. Direct microscopy results were expressed not as ”positive” or negative” but on a scale from 0 to 4 where 0 represents ”convincingly negative” and 4 ”convincingly positive”.

30 patients had bone marrow or spleen aspirations and promastigote cultures done. Most patients have all relevant records for this retrospective analysis. One interruption in the supply of SSG occurred with increased deaths. In other times a dramatic response to the SSG was used as a fourth diagnostic criteria. 23 patients had positive promastigote cultures and 7 were negative. Of 22 promastigote culture positives with RDT records 6 were negative by RDT, giving 73% sensitivity. 29 patients had RDT records, 7 were negative and 22 were positive. Of these 7 RDT negatives 6 were positive by promastigote culture.

With ”real world stains, microscopes, specimens and microscopist” all 7 direct microscopies with all 7 RDT negative patients for amastigotes were inconclusive, i.e. not clearly positive or negative for amastigotes.

Conclusion: Promastigote culture with simple house made culture media is feasible in very low resources setting and is very useful with rK39 RDT negative patients. In our hands direct microscopy for amastigotes did not contribute anything for RDT negative patients.

References:
WHO Leishmaniasis Country Profiles Somalia 2008—www.who.int/leishmaniasis/resources/SOMALIA.pdf
WHO Visceral Leishmaniasis Rapid Diagnostic Test Performance—www.who.int/tdr/publications/documents/vl-rdt-evaluation.pdf

Correspondence: Email - mikko_aalto@yahoo.com

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Refugee Migration and Cutaneous Leishmaniasis, in Turkey

Nebiye Yentur Doni [1,2*].

[1] Harran University Vocational School of Health Services Department of Medical Microbiology, Sanliurfa, Turkey; [2] The Ohio State University, Wexner Medical Centre, Department of Pathology and Microbiology, Columbus, Ohio, USA.

Available Online: 30 December 2018

ABSTRACT

Background: By the beginning of 2018, 3.572.565 Syrians had fled Turkey since the civil war began in Syria in 2011, due to open border policy of Turkish government. Of these, 224.334 take shelter in temporary refugee camps, while 3.348.231 remain living outside of refugee camps [1]. War and conflicts cause the re-emergence of infectious diseases including cutaneous leishmaniosis (CL) as a result of collapsed health care infrastructures and population migration. When the refugees migrate to new places they might be exposed to new infections, and introduce and transmit their diseases to new areas and, populations. Cutaneous leishmaniosis is the most common form of the leishmaniosis, responsible for about three-fourths of the total cases globally [2]. Although CL has been well known for many decades in Turkey , an increasing prevalence of CL is seen in both endemic and non -endemic areas in correlation with the Syrian refugee influx. In light of these data we aimed to evaluate the effects of Syrian conflict on CL.

Methods: The data presented in this study are gathered primarily from the reports written after the Syrian civil war began (2011), about the causative agents of cutaneous leishmaniasis among Syrian refugees in Turkey. The Cochrane Library, MEDLINE, Embase, and US National Library of Medicine National Institutes of Health; Web of Science, Web sites of World Health Organization, United Nations Refugee Agency, and the Health Ministry of Turkey were searched with the terms 'cutaneous leishmaniosis' 'Turkey' 'Syria' or 'Syrian' and 'migration,' and 'refugee'.

Results: Turkey is an endemic country for CL and south/south eastern regions of Turkey are already hyper endemic regions that CL cases are mostly reported in. However, CL is not limited to these regions owing to increases of Syrian CL cases and permanent or seasonal migrations of CL cases to nonendemic areas. Cutaneous leishmaniosis cases have also been reported from the Central Anatolia and Ege Regions. *Leishmania tropica* (*L.tropica*) is the major causative agent of CL; however *Leishmania infantum* is the causative agent in the eastern Mediterranean region of Turkey [3]. It has been reported that *L. tropica* is detected among Syrian refugees [4-8] and, non-endemic parasite strains *Leishmania major* and *Leishmania donovani* were introduced by incoming refugees [5, 7]. In a study that investigated the dermatological infectious diseases, CL was the most diagnosed among Syrian patients [6].

Conclusions: This study presented once again that, there has been dramatical increase in CL incidence in recent years in Turkey, both in terms of clinical cases and CL parasite strains, which might be related with continuing influx of Syrian refugees. It should be kept in mind that when prevention and control methods are ignored, the considerable influx of CL cases, and the sand flies which transmit CL cases might spread CL and cause an outbreak.

Keywords: Cutaneous leishmaniasis, Syrian refugees, Turkey

References:

1] GİGM. Geçici Koruma T.C. İçişleri Bakanlığı Göç İdaresi Genel Müdürlüğü. 2018. http://www.goc.gov.tr/icerik6/gecici-koruma_363_378_4713_icerik. Accessed 04/09/ 2018.
2] WHO. Leishmaniasis Fact sheet N°375. 2017. http://www.who.int/mediacentre/factsheets/fs375/en/. Accessed 11/10 2017.
3] Ozbilgin A, Harman M, Karakus M, Bart A, Toz S, Kurt O et al., Acta Trop. 173 (2017) 90-96. doi:10.1016/j.actatropica.2017.05.032
4] Eksi F, Ozgoztasi O, Karsligil T, Saglam M., J Int Med Res. 45 (2017) 1 114-122. doi:10.1177/0300060516677155
5] Gurses G, Ozaslan M, Zeyrek FY, Kilic IH, Doni NY, Karagoz ID et al., Folia Microbiol (Praha) (2017). doi:10.1007/s12223-017-0556-1
6] Inci R, Ozturk P, Mulayim MK, Karakuzu A, Kelekci KH, Inci MF et al., Turkderm-Turk Arch D 50 (2016) 4 145-149. doi:10.4274/turkderm.62443
7] Koltas IS, Eroglu F, Alabaz D, Uzun S., Trans R Soc Trop Med Hyg. 108 (2014) 3 154-158. doi:10.1093/trstmh/trt119
8] Ozkeklikci A, Karakus M, Ozbel Y, Toz S., Acta Trop. 166 (2017) 166 35-38. doi:10.1016/j.actatropica.2016.10.019

Correspondence: Email - n_doni@hotmail.com

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Target-based drug design and development of drug delivery systems to tackle leishmaniasis

Nicola Micale [1*], Angela Scala [1] and Anna Piperno [1].

[1] Laboratory of Nanomedicine, Biomaterials and Heterocyclic Compounds - Department of Chemical, Biological, Pharmaceutical and Environmental Sciences - University of Messina, Viale F. Stagno D'Alcontres 31, I-98166 Messina (ITALY)

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis is one of the most neglected, overlooked and deadly vector-borne infectious diseases afflicting primarily people of tropical and sub-tropical areas of the developing countries. Chemotherapy is currently the only effective approach to treat all forms of leishmaniasis. However, its effectiveness is severely limited due to high toxicity, long treatment length, drug-resistance or inadequate mode of administration. An effective vaccine for humans has not been developed yet. This scenario created an urgent and continous need for new interventions. In our ongoing search in this field, we identified new molecular scaffolds (i.e. decorated fused benzo[*d*]thiophene [1], enolizable cyclic β,β′-triketone [2] and 1,2-substituted-1*H*-benzo[*d*]imidazole [3]) targeting *Leishmania mexicana* CPB2.8ΔCTE, one of the more promising targets for antileishmanial drug design. Moreover, we successfully developed new delivery systems based on biocompatible polymeric backbones such as PLGA-PEG copolymer [4] and hyaluronic acid (HA) [4,5] containing the antileishmanial drug pentamidine (Pent). The novel PLGA-PEG-Pent and HA-Pent bioconjugates have been proposed, respectively, as non-targeted and targeted drug delivery systems.

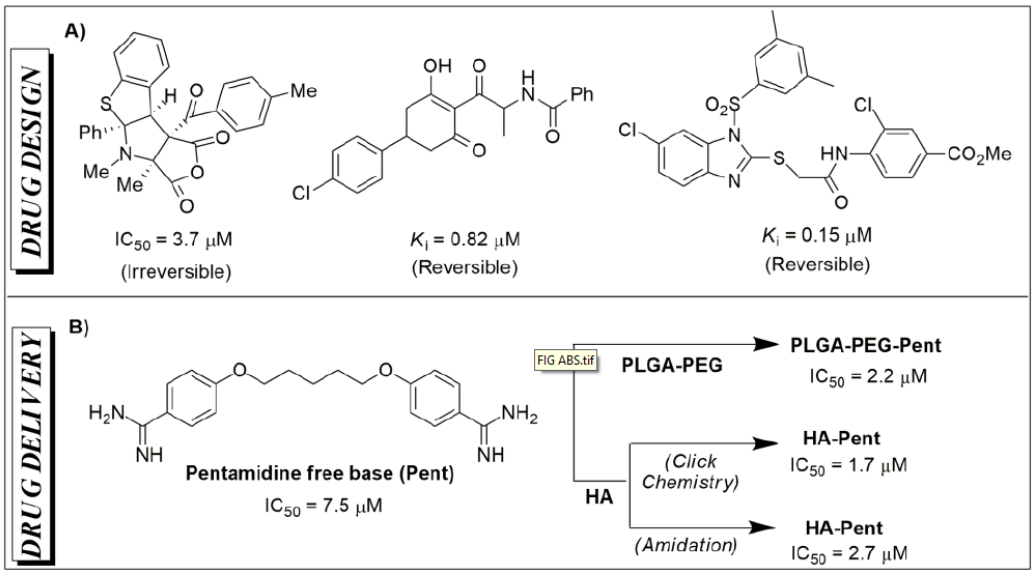

Figure 1 | Identified lead structures targeting *L. mexicana* CPB2.8ΔCTE; B) Pentamidine bioconjugates.

Correspondence: Email - nmicale@unime.it

References:

1] A. Scala, N. Micale, A. Piperno, et al., RSC Adv., 6 (2016) 30628-30635. doi:10.1039/C6RA05557E
2] A. Scala, A. Rescifina, N. Micale, A. Piperno, et al., Chem. Biol. Drug Des., 91 (2018) 597-604. doi:10.1111/cbdd.13124
3] N. Micale, et al., Chem. Biol. Drug Des., submitted (2018)
4] Scala, A. Piperno, N. Micale, et al., J. Biomed. Mater. Res. B, (2017). doi: 10.1002/jbm.b.34058
5] N. Micale, A. Piperno, A. Scala, et. al., RSC Adv., 5 (2015) 95545-95550. doi:10.1039/C5RA18019H

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Sand fly salivary proteins and their potential use in the control of canine leishmaniasis

Petr Volf [1]*, Tereza Lestinova [1], Tatiana Spitzova [1], Laura Willen [1].

[1] Dept. Parasitology, Faculty of Science, Charles University, Prague, Czech Republic

Available Online: 30 December 2018

ABSTRACT

During *Leishmania* transmission, sand flies inoculate the host with saliva composed of a diverse group of molecules (proteins and peptides) which have anti-hemostatic, anti-inflammatory and immunomodulatory properties (reviewed in [1]). *Leishmania* parasites benefit from a saliva-altered local immune reaction. In naive hosts, saliva exacerbates the infection, causing "enhancing effects", reflected by larger lesions and higher parasite numbers [2]. In contrast, hosts repeatedly exposed to bites of uninfected sand flies or immunized by salivary proteins were usually protected against *Leishmania* infection [3, 4]. Therefore, sand fly salivary antigens are proposed as vaccine candidates against leishmaniasis.

Hosts repeatedly bitten by sand flies also develop specific anti-saliva antibodies. Levels of anti-saliva IgG reflect the intensity of exposure to sand flies and thus can be used in epidemiological studies, e.g. to measure the effectiveness of vector-control campaigns or as a marker of risk for *Leishmania* transmission (reviewed in [1]). Around the Mediterranean basin, the sand fly Phlebotomus perniciosus is the principle vector of *Leishmania infantum*, the causing agent of canine leishmaniasis. A salivary Yellow protein SP03B of P. perniciosus has been shown as a valid biomarker to estimate dog exposure to *P. perniciosus* [5, 6]. Since standard serological methods are impractical and time-consuming in field conditions, we developed the rSP03B sero-strip, a rapid test that can be immediately applied to screen large cohorts of dogs for the presence of anti-*P. perniciosus* antibodies. It is highly sensitive and specific and shown to be a valid replacement for standard ELISA assays. It is the first rapid test designed for medical entomology research and it can be employed during epidemiological studies of canine leishmaniasis in the Mediterranean area.

Acknowledgments:
This work was supported by project CePaViP (CZ.02.1.01/0.0/0.0/16_019/0000759), the Czech Science Foundation (17-10308S) and Research Centre UNCE 204072 and has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement N° 642609.

References:
1] Lestinova T, Rohousova I, Sima M, de Oliveira CI, PLoS Negl Trop Dis. 11 (2017) 7. doi:10.1371/journal.pntd.0005625
2] Titus RG, Ribeiro JMC, Science 239 (1988) 4845 1306-1308. doi:10.1126/science.3344436
3] Kamhawi S, Belkaid Y, Modi G, Rowton E, Sacks D., Science. 290 (2000) 5495 1351-1354. doi:10.1126/science.290.5495.1351
4] Valenzuela JG, Belkaid Y, Garfield MK, et al., Journal of Experimental Medicine 194 (2001) 3 331-342. doi:10.1084/jcm.194.3.331
5] Kostalova T, Lestinova T, Sumova P, Vlkova M, et al., PLoS Negl Trop Dis. 9 (2015) 6. doi:10.1371/journal.pntd.0003832
6] Kostalova T, Lestinova T, Maia C, Sumova P, et al., Med Vet Entomol 31 (2017) 1 88-93. doi:10.1111/mve.12192

Correspondence: Email - volf@cesnet.cz

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Feral cats as a main reservoir of Leishmania in South of Spain

Sandra López Fernández [1*], Carmen Chicharro [2], Eugenia Carrillo [2], Eduardo Martínez Manzanares [1], Javier Moreno Nuncio [2]

[1] Universidad de Málaga, Spain; [2] Instituto de Salud Carlos III

Available Online: 30 December 2018

ABSTRACT

The latest studies have showed that domestic cats can be a chief reservoir also, mainly these animals injured by a retroviral diseases like Feline Leukemia (FeLV) or Felines Immunodeficiency (FIV). Both retrovirus proliferate out between cat who lives nearby, in territorial fights, using same drink or feeding points. Based on these studies, it can be expected that outdoor cats which come from settled colonies will show high incidence of leishmanial infantum. At least, it has been hope find animals with antibodies against LI. A hundred of feral cats were tested by qualitative serology assay for Leishmaniosis and retroviral diseases (check by IFA, ELISA and PCR).

Seroprevalence in stray cats were 28,5% and it were founded one contradiction between other publications about the comorbility with retroviral diseases like FeLV or FIV. In this study 14,3% of cats showed antileishmania-antibodies, nevertheless only 3,6% were FeLV positives at the same time than Leishmaniasis and 7,1%were also FIV positives with leishmaniasis. PCR isolated Leishmania DNA in 30.4% of tested cats. Stray cats can play more important fact in leishmaniosis transmission than it were suspected.

Acknowledgments:
Javier Moreno Nuncio, Eduardo Martínez Manzanares and Fernando Fariñas Guerrero, for their invaluable support and help along this years, and believe in my work.

References:
1] Pennisi MG, Miro G. et Al, Parasit vectors 8 (2015) 302. doi:10.1186/s13071-015-0909-z
2] Maroli M, Pennisi MG, Di Muccio T, Khoury C, Gradoni L, Gramiccia M., Vet Parasitol 145 (2007) 357–360. doi:10.1016/j.vetpar.2006.11.009
3] Pennisi MG., Vet Parasitol 208 (2015) 35–47. doi:10.1016/j.vetpar.2014.12.023

Correspondence: Email - sandralopezfernandez@uma.es

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Whole-genome sequencing of Trypanosomatidae clinical isolates reveals *Crithidia*-like species in cases of visceral and cutaneous leishmaniasis in Brazil

Sandra R. Maruyama [1*], Alynne K. M. de Santana [2], Cristiane M. Milanezi [3], Caio A. B. Oliveira [1], Amélia R. Jesus [2] , Aline S. Barreto [2], Angela M. da Silva [2], Roque P. Almeida [2], José M. Ribeiro [4], João S. Silva [3,5].

[1] Federal University of São Carlos, São Carlos, SP, Brazil; [2] University Hospital, Federal University of Sergipe, Aracaju, SE, Brazil; [3] University of São Paulo, Ribeirão Preto, SP, Brazil; [4] National Institute of Allergy and Infectious Diseases, NIAID-NIH, Rockville, MD, USA; [5] Fiocruz- Bi-Institutional Translational Medicine Project, University of São Paulo, Ribeirão Preto, SP, Brazil.

Available Online: 30 December 2018

ABSTRACT

Over the last decades, sporadic reports of coinfections of *Leishmania* with apparently monoxenous trypanosomatids (or monoinfection) have been described (revised by Kaufer et al [1]). The question arises as to whether these unusual infections are occasional findings or are evidence for novel parasites with the potential to threaten public health. Through DNA sequence analysis of parasite strains isolated from patients presenting both visceral (VL) and cutaneous leishmaniasis (CL) symptoms, we identified a new pathogenic trypanosomatid in 33 out of 34 clinical cases diagnosed as VL and in one case of CL in Brazil. The confirmation of a new species was achieved by whole-genome sequencing analyses of seven clinical isolates. By comparing coding sequences of orthologous genes (ranging from 1000 to over 6000 genes) within 36 Trypanosomatidae organisms, we constructed a phylogram based on a total orthologs median matrix (TOMM) and found that the new parasite is closely related to *Crithidia fasciculata*, which parasitizes exclusively mosquitoes and is considered noninfective to humans. Our findings raise concerns about an emerging infectious disease that is easily confused with leishmaniasis, opening a research path to address epidemiological questions about the identification of vectors, reservoirs, and distribution patterns; the reassessment of leishmaniasis cases in Brazil; better methods for parasite species identification; and drug resistance and new treatment options.

Acknowledgments:
This work was supported by grants from the São Paulo Research Foundation–FAPESP, under agreements 2016/20258-0 (Young Investigator Award) and 2013/08216-2 (Center for Research in Inflammatory Diseases), and by CAPES grant number 23038.005304/2011-01 and CNPq grant number 552721/2011-5. SRM received a fellowship from FAPESP (2017/16328-6).

References:
1] Kaufer A, Ellis J, Stark D, Barratt J. The evolution of trypanosomatid taxonomy. Parasites & Vectors. 2017;10:287.

Correspondence: Email - srmaruyama@gmail.com

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Sand flies, mycobiota and leishmaniasis

S Epis [1*], E Martin [1], I Varotto Boccazzi [1], L De Marco [2], L Gradoni [3], N Basilico [4], L Sacchi [5], C Bandi [1].

[1] University of Milan - Department of Biosciences and Pediatric Clinical Research Center; [2] University of Camerino - School of Biosciences and Veterinary Medicine; [3] Istituto Superiore di Sanità - Unit of Vector-Borne Diseases and International Health; [4] University of Milan - Department of Biomedical Sciences, Surgical and Dental; [5] University of Pavia - Department of Biology and Biotechnology.

Available Online: 30 December 2018

ABSTRACT

The use of yeasts in biological control is limited, probably due to the fact that yeasts are generally regarded as organisms that exert a positive effect on insects, e.g. a nutritional role. However, beyond the nutritional function, there are several other roles that have been suggested for yeasts, but with limited experimental evidences: one of these is the protective or antagonist role of the yeast symbionts against pathogenic microorganisms. Here, we focused our research on the sand fly *Phlebotomus perniciosus*, the main vector of human and canine leishmaniasis in the Mediterranean area, with the intentions of describing the mycobiota and of investigating if yeasts associated with this insect could exert inhibitory/killing activity against the pathogen *Leishmania* spp. First, we investigated the mycobiota by culture-dependent approaches (microbiological analyses and the sequencing of the 26S rRNA), ITS rRNA metagenomic analysis, fluorescence in situ hybridisation (FISH) and phylogenomic analysis [1]. Second, we focused our attention on *Wickerhamomyces anomalus*, an ascomycete yeast well known for its antimicrobial properties; this yeast was phylogenetically characterized and tested against selected yeast strains, proving its killer phenotype [2; 3]. Finally, we tested the *in vitro* activity of *W. anomalus* strains against the pathogen *Leishmania infantum.* in order to explore their potential inhibitory/killing property against pathogens. We believe that this study offers the basis for the development of an environmental-friendly and safe "tool" to interfere with sand fly vectorial capacity, which may be included in the integrated approaches for the control of leishmaniasis, a worldwide re-emerging public health problem.

References:

1] E. Martin, I. Varotto Boccazzi, L. De Marco, G. Bongiorno, M. Montagna, L. Sacchi, P. Mensah, I. Ricci, L. Gradoni, C. Bandi, S. Epis, Environ. Microbiol 20 (2018) 3 1064–1077. doi:10.1111/1462-2920.14044.

2] E. Martin, G. Bongiorno, L. Giovati, M. Montagna, E. Crotti, C. Damiani, L. Gradoni, L. Polonelli, I. Ricci, G. Favia, S. Epis, Med. Vet. Entomol 30 (2016) 1 101–106

3] A. Cappelli, U. Ulissi, M. Valzano, C. Damiani, S. Epis, M.G. Gabrielli, S. Conti, L. Polonelli, C. Bandi, G. Favia, I. Ricci, PLoS One 9 (2014) 5. doi:10.1371/journal.pone.0095988

Correspondence: Email - sara.epis@unimi.it

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Molecular epidemiology and the investigation of *Leishmania* RNA virus of *Leishmania tropica* strains in Turkey

Muhammed Nalçaci [1], Mehmet Karakuş [2], Bahtiyar Yilmaz [1], Samiye Demir [1], Yusuf Özbel [3], Seray Töz [3] .

[1] Ege University, Institute of Science, Division of Biology, Department of Zoology , Bornova, İzmir, Turkey; [2] University of Health Sciences, Department of Biotechnology, Istanbul, Turkey; [3] Ege University, Medical Faculty, Department of Parasitology, Bornova, İzmir, Turkey.

Available Online: 30 December 2018

ABSTRACT

In Turkey, cutaneous leishmaniasis (CL) is a major public health threat and causative agent is mainly *Leishmania (L.) tropica*. We aimed to evaluate the situation of *L.tropica* in Turkey by multilocus microsatellites typing (MLMT) besides *Leishmania* virus (LRV) positivity for better understanding the epidemiology and virulence of the parasite.

Leishmania isolates/slide samples obtained from CL patients were analyzed by MLMT. MLMT was performed with 12 highly specific microsatellite markers using genomic DNA. Repeat numbers for each locus were recorded and Delta K (ΔK) calculation, and Bayesian statistics were used for determining the population structure. Delta K calculations were showed three main populations (POP-A, POP-B, POP-C). Further analysis was revealed three subpopulations for POP-B and POP-C, while no subpopulation was identified for POP-A. One of *L. tropica* strain was viscerotropic, while the rest were dermatropic. Descriptive analysis per populations (K:3) was also performed and POP-B was found to be the most heterogeneous group by having the highest number of alleles (3.00) and H_e (0.466) values and H_o (0.139) values and the mean number of alleles per populations was 2.3.

Additionally, the presence of LRV was investigated in 24 *L.tropica* isolates using agarose gel method after genomic DNA extraction and PCR from cDNA samples after RNA extraction. Positives were sequenced by Sanger method. Seven *L.tropica* strains were found to be positive only in PCR and sequence analyses showed similarities with LRV2.

Our findings revealed that understanding the epidemiological origin of the parasite and virus positivity could be possible using different kind of clinical samples for diagnosis and thus will give a better chance to evaluate the clinical status of the patient by parasitologists and clinicans. Data available here will provide epidemiological knowledge for further studies.

Acknowledgments:
This study was supported by TÜBİTAK Project No:114S999 and Ege University Funds (BAP) with Project no:17-FEN-045.

Correspondence: Email - seray.ozensoy.toz@ege.edu.tr

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Synthetic peroxides as potential anti-Leishmania chemotypes

S Cortes [1*], Andreia Mendes [1], Lilia Cabral [2], M L S Cristiano [2], L Campino [1]

[1] Global Health and Tropical Medicine Center, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, Rua Junqueira, 100, 1349-008 Lisbon, Portugal; [2] CCMAR and Departamento de Química e Farmácia, Faculdade de Ciências e Tecnologia, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal

Available Online: 30 December 2018

ABSTRACT

Leishmania infantum is the causative agent of human and canine leishmaniasis in the Mediterranean basin. The few available drugs show toxic side effects, declining efficacy with increasing drug resistance and are expensive. Artemisinin and its derivatives (ARTs) feature among the most important classes of antimalarial agents. ARTs and related synthetic peroxides have demonstrated high efficacy against Plasmodium sp. and other parasites such as Perkinsus sp. and *Toxoplasma gondi* [1-3].

Previous results of our team have shown interesting *in vitro* activity of a small library of peroxides against *L. infantum* parasites [4]. We are expanding our investigations by evaluating the cytotoxicity and susceptibility of *L. infantum* and *L. donovani* promastigotes to an extended collection of peroxides comprising artemisinin-derived trioxanes and synthetic trioxolanes, using anti-*Leishmania* drugs currently in use as controls. Inhibitory concentrations (IC50s) were calculated and the cytotoxicity of each compound was accessed in the J774A.1 cell line.

Results have shown that the parasites are susceptible to the peroxides tested, at IC50s ranging between 13 μM and 491 μM for both *Leishmania* species. Moreover, our results have shown that the activity of this chemotype is dependent on the peroxide group. The encouraging results obtained for the activity and safety of trioxolanes, together with their easy access through chemical synthesis, support the relevance of further studies in the context of leishmanial therapy. Susceptibility of the macrophage-amastigote system and possible modes of action for this group of compounds are being explored.

Acknowledgments:
This work was supported by funds from Fundação para a Ciência e a Tecnologia (FCT), Ministério da Ciência, Tecnologia e Ensino Superior through GHTM (UID/Multi/04413/2013) and CCMAR (UID/Multi/04326/2013 and PTDC/MAR-BIO/4132/2014). S.Cortes holds a FCT Investigator Starting Grant IF/0773/2015.

References:
1] I. Copple, A. Mercer, J. Firman, G. Donegan, B. Herpers, M. H. L. Wong, J. Chadwick, A. D. Bringela, M. L. S. Cristiano, B. v. d. Water, S. A. Ward, P. M. O'Neill, B. K. Park, Mol Med 18 (2012) 1045-1055. doi:10.2119/molmed.2012.00154
2] N.C.P. Araujo, R. Afonso, A. Bringela, M.L. Cancela, M.L.S. Cristiano, and R.B. Leite, Parasitol Int 62 (2013) 575-582. doi:10.1016/j.parint.2013.06.010
3] Loo et al., Pharmacol Res. 117 (2017) 192–217. doi:10.1016/j.phrs.2016.11.012
4] S. Cortes, A. Albuquerque, L.I.L. Cabral, L. Lopes, L. Campino, and M.L.S. Cristiano, Antimicrob Agents Chemother 59 (2015) 5032–5035. doi:10.1128/AAC.00298-15

Correspondence: Email - scortes@ihmt.unl.pt

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

An Analysis of Hematological and Biochemical Parameters of cats infected by *Leishmania infantum*

Trícia Maria Ferreira de Sousa Oliveira [1,2*], Diogo Tiago da Silva [2], Maria Luana Alves [2], Julio César Pereira Spada [2], Julia Cristina Benassi [1], João Augusto Franco Leonel [2], Nuno Wolfgang Pereira [2], Wilma Aparecida Starke-Buzetti [3]

[1] Departamento de Medicina Veterinária, FZEA, USP, Pirassununga, SP, Brasil; [2] Programa de Pós-graduação em Epidemiologia Experimental Aplicada às Zoonoses, FMVZ, USP, Pirassununga, SP, Brasil; [3] Departamento de Biologia e Zootecnia, UNESP, Ilha Solteira, SP, Brasil

Available Online: 30 December 2018

ABSTRACT

In this study we aimed a clinical evaluation and comparative analysis between hematological and biochemical parameters of cats with and without *Leishmania infantum* infection. The cats were separated in two groups: group 1 (G1, n = 5), cats naturally infected by *L. infantum*, diagnosed by parasitological, serological and molecular tests (including sequencing of positive PCR products) group 2 (G2, n = 6) uninfected cats, negative by parasitological, serological and molecular tests. Samples of blood collected directly from the jugular vein were prepared for hematological and biochemical exams, described in Table 1. The mean of each parameter was compared between the experimental groups (G1 and G2) by the t-Student test1. In the clinical evaluation, we observed that 100% (5/5) of the cats infected by *L. infantum* presented thinness (body score 1), 80% (4/5) had alopecia and lesions on the body (skin, snout or ear), 40 % (2/5) contained hypertrophic lymph nodes and one animal had conjunctivitis. All negative cats were asymptomatic. Regarding hematological examinations we detected a reduction of platelets ($p = 0.0062$, $p < 0.05$), increase of erythrocytes ($p = 0.0063$, $p < 0.05$) and PPT concentration ($p = 4.4832e-06$, $p < 0.05$) in G1 cats compared to G2, whereas leukocytes were increased for G2 cats ($p = 0.014$, $p < 0.05$). By the biochemist, the infected cats showed a reduction in the concentration of albumin and aspartate aminotransferase ($p = 0.0065$ and $p = 0.0025$, $p < 0.05$, respectively). The hematological and biochemical data herein described shows an association between *L. infantum* infection and platelet reduction and hyperproteinemia as well as described for canine visceral leishmaniasis. In enzootic areas, significant alterations described, can be suggestive to *L. infantum* infection in cats and specific tests should be recommended.

Acknowledgments:
Fundação de Amaparo à Pesquisa do Estado de São Paulo (FAPESP) grant. 2016/08018-4

References:
1] R Core team. R: A language and enviroment for estatistical computing. R foundation for statistical computing. Viena, Austria. ISBN 3-900051-07-0 (2016)

Correspondence: Email - tricia@usp.br

Table 1 | Comparison between Hematological and Biochemical Parameters of cats infected and non-infected by *Leishmania infantum*.

Parameters	Reference range	Infected	Non Infected	*p*- value
Red blood cells (10^6/µL)	5 - 10	8,07 ± 0,51	7,03 ± 0,46	$p < 0,01$
HCT (%)	24 - 45	33,76 ± 3,21	32,26 ± 2,30	0,3927
Hemoglobin (g/dl)	8 - 15	10,14 ± 0,69	10,36 ± 0,24	0,4708
MCV (fl)	39 - 55	44,32 ± 4,29	46,00 ± 4,49	0,5444
MCHC (%)	30 -36	30,1 ± 0,87	32,26 ± 2,64	0,1154
Platelet (10^3/µL)	230 - 680	122,4 ± 16,07	265,5 ± 88,25	$p < 0,01$
White blood cells (10^3/µL)	5,5 -19	13,78 ± 4,09	21,25 ± 4,03	$p < 0,05$
Eosinophil (10^3/µL)	0 -1,5	0,98 ± 0,50	1,73 ± 0,22	0,06332
Neutrophil (10^3/µL)	2,5 - 12,5	10,17 ± 3,37	14,15 ± 2,21	$p < 0,05$
Lymphocyte (10^3/µL)	1,5 - 7	2,58 ± 1,04	5,11 ± 2,54	$p < 0,05$
Monocyte (10^3/µL)	0 - 0,85	0,40 ± 0,36	0,25 ± 0,24	0,4293
Urea (mg/dl)	42,8 - 64,2	97,6 ± 16,51	97 ± 14,01	0,9494
Creatinine (mg/dl)	0,8 - 1,8	1,04 ± 0,21	1,46 ± 0,36	0,05226
ALP (U/L)	7 - 80	15,78 ± 1,56	13,97 ± 2,50	0,1957
ALT (U/L)	06 - 83	36 ± 12,94	39,5 ± 20,63	0,7506
AST (U/L)	26 - 43	21,6 ± 4,97	38 ± 7,56	$p < 0,01$
Total protein (g/L)	54 - 78	92,6 ± 3,28	72,5 ± 3,50	$p < 0,01$
Albumin (g/dl)	2,1 - 3,3	1,54 ± 0,11	2,26 ± 0,44	$p < 0,01$

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Canine visceral leishmaniasis: serological and molecular diagnosis of dogs infected with *Leishmania infantum* in Brazil

V L R da Matta [1]*, L F S Batista [1], M D Laurenti [1], R M Silva [2], J E Tolezano [2], H R L R de Lucca [2]

[1] Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (LIM50)- Brasil; [2] Instituto Adolfo Lutz de São Paulo e Rio Claro- Brasil.

Available Online: 30 December 2018

ABSTRACT

In countries where visceral leishmaniasis (VL) is a serious public health problem, such as in Brazil, the identification of infected dogs and their succeeding removal from the endemic area is essential to VL control, since dogs are the main reservoir of the zoonotic VL and capable to transmit *Leishmania infantum* to the vector efficiently[1]. Thus, to assure a reliable diagnosis of infected dogs, the Brazilian Ministry of Health recommends the sequential use of two serological tests. Up to December 2011, ELISA was the screening method and the Indirect Immunofluorescence Assay (IFA) the confirmatory one (old protocol). After that, it was introduced a quick assay accomplished in a Dual-Path Platform (DPP) followed by ELISA (new protocol). Herein, we compared the performance of both protocols using serum samples obtained during a cross-sectional investigation in 2010 (n= 186) and in 2012 (n=856) in an endemic area for VL in southeast Brazil. The current protocol estimated lower prevalence rates of canine infection than did the old protocol, both in 2010 (3.8% x 9.7%) and in 2012 (0.5% x 4.4%). The low concordance of ELISA with the positive DPP results contributed to a considerable decay of the final prevalence (Fig 1). We understand that one of the main forces related to this disagreement relies on sera reactivity to different parasite antigens, i.e, with *L. infantum* recombinant fusion protein rK28 (k9+ k39+ k26) of the DPP, and with total soluble proteins of *L. major*-like in the ELISA. The same did not occur between ELISA and IFA (old protocol), both based on *L. major*-like reactivity. Of note is that DPP did not show any cross-reactivity with sera (n=22) of dogs with other pathologies, but ELISA partially reacted with sera of dogs with neosporosis, babesiosis, ehrlichiosis, and Chagas disease. In this regard, our previous study with a well-defined population consisting of dogs with proven *Leishmania* infection showed that DPP presented higher specificity (95.1% x 77.8% x 69.1), positive predictive value (95.1% x 81.1% x 76.6%) and positive likelihood value (18.3% x 4.1% x 3.1) in comparison to ELISA and IFA, respectively. Besides, DPP detected both asymptomatic and symptomatic dogs in equal proportions [2]. Alternatively, PCR with non-invasive samples seems to be a good tool for the canine VL diagnosis. In a concomitant study, conjunctival and buccal swabs from infected dogs (n= 58), when processed together, yielded a positivity rate equivalent to lymph node, and above of that found in blood in symptomatic dogs, reinforcing findings of our previous investigation performed in another endemic area for VL [3]. In conclusion, the current Brazilian serological protocol, that now includes the DPP, presents good accuracy, and has potential to misdiagnosis fewer dogs than the previous one, principally by reducing false-positive results. Respective to the molecular diagnosis, the effective detection of *Leishmania* in canine non-invasive samples is quite relevant for the differential VL diagnosis with other diseases that provoke overlapping signs and symptoms.

Acknowledgments:

Supported by grants #2014/50315-0, #2012/50285-9 from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Brazil; # 404158/2016, # 476479/2012-6 from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Brazil, and Instituto Adolfo Lutz.

References:

1] MD Laurenti, CN Rossi, VLR da Matta, et. al., Vet Parasitol 196 (2013) 3-4 296-300. doi:10.1016/j.vetpar.2013.03.017
2] MD Laurenti, M.V. de Santana L.Jr., TY Tomokane, HRL de Lucca, M. Aschar, CSF Souza, RM Silva, M Marcondes, VLR da Matta, Vet Parasitol, 205 (2014) 3-4 444-450. doi:10.1016/j.vetpar.2014.09.002
3] M Aschar, ET de Oliveira, MD Laurenti, M Marcondes, JE Tolezano, RM Hiramoto, CE Corbett, da Matta VLR. Vet Parasitol, 225 (2016) 108-113. doi:10.1016/j.vetpar.2016.06.005

Correspondence: Email - mattav@usp.br

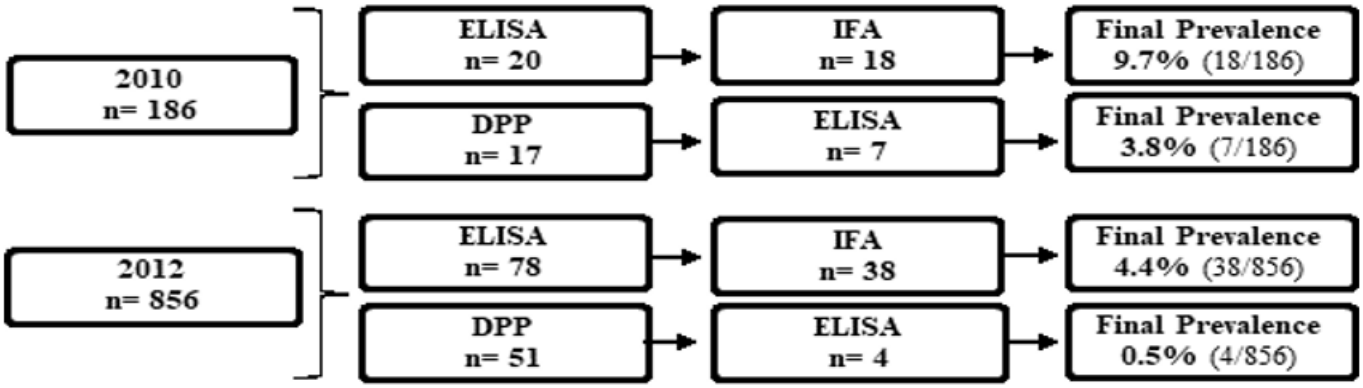

Figure 1 | Prevalence of canine infection in endemic area for VL based on two Brazilian serological protocols (ELISA/IFA, DPP/ELISA)

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Canine Visceral Leishmaniasis as a fibrotic disease

Wagner Luiz Tafuri [1*], Adriano Francisco Alves [1], Izabela Ferreira Gontijo Amorim [1]; Ramon Alencar Pereira [1]; Igor Matheus Valadares Mendes Madeira [1]; Rodrigo S. Castro Lucélia Coimbra [1]; Washington Luiz Tafuri [2] ‡

[1] Departamento de Patologia Geral, Instituto de Ciencias Biológicas, Universidade Federal de Minas Gerais; [2] Departamento de Anatomia Patologica, Faculdade de Medicina, Universidade Federal de Minas Gerais; ‡ - in memorian.

Available Online: 30 December 2018

ABSTRACT

We propose that Canine Visceral Leishmaniasis (CVL) is a systemic fibrotic disease, as evidenced by the wide distribution of fibrosis that we have found in the dogs suffering from chronic condition. The inflammatory cells apparently direct fibrosis formation. We have described an intense collagen deposition (fibropoiese) in livers, spleen, cervical lymph nodes, lung and kidney of all dogs naturally and experimentally infected with *Leishmania (L.) infantum*. In fact, all infected dogs showed higher numbers of reticular fibers than controls independently of the clinical status (besides fibropoesis seems to be more prominent in symptomatic dogs). Thus, we have investigated some aspects of this question by analyzing the expression pattern of different known fibrosis markers in distinct canine organs for example: (1) in livers positive correlation has been found among fibropoesis, chronic granulomatous reaction, parasite tissue load and expression of smooth muscle alpha-actin (α-SMA) (a superior marker for activated HSC cells than vimentin and cytokeratin); (2) in lungs the main histopathological picture is chronic and diffuse interstitial pneumonitis. The thickened interalveolar septa is characterized by the cellular exudate (mostly plasma cells, macrophages and lymphocytes) associated with collagen deposition type III (reticulin) and I. Myofibroblasts were characterized by immunohistochemistry based on α-SMA, vimentin, cytokeratin, E-cadherin, Snail antigen homolog 1 (SNAIL-1), and transforming growth factor-beta (TGF-β). All myofibroblasts markers are higher in naturally infected dogs compared to uninfected dogs; (3) in kidneys animals has been arevealed glomerular and interstitial fibropoiesis associated with different types of glomerulonephritis and chronic interstitial nephritis. As seen in livers and lungs, myfibroblasts markers as α-SMA and vimentin are notable in both glomerulus and tubulointersticial fibrosis process. The expression of the cytokine TGF-beta was also higher than uninfected dogs. Thus, we have concluded that mesenchymal cells are active in promoting changes in the extracellular matrix in all studied organs of dogs naturally infected with canine visceral leishmaniasis reporting a systematic fibrotic picture.

Acknowledgments:
The authors thank FAPEMIG for grant APQ-01378-12, CNPq for grant 474665/2012-7 and grant 303022/20132.

References:
1] CM Keenan et al., Vet. Pathol. 21 (1984) 80–86. doi:10.1177/030098588402100113
2] T. Rallis T et al., J. Comp. Pathol. 132, 145–152, 2005.
3] F. MELO, et. al., Am. J. Trop. Med. Hyg. 79, 198-204. 2008.
4] F. MELO, et. al., Int. J. Exp. Pathol. 90, 538-548. 2009. doi:10.1111/j.1365-2613.2009.00681.x
5] LC. SILVA, et al., Int. J. Exp. Pathol. 94, 133–143. 2013. doi:10.1111/iep.12029
6] IM, Madeira, et al., Int J Exp Pathol. 97: 139-149; 2016. doi:10.1111/iep.12189
7] RS Castro, Vet Parasitol. 30: 250:22-29, 2018. doi:10.1016/j.vetpar.2017.12.005

Correspondence: Email - wagnertafuri@gmail.com

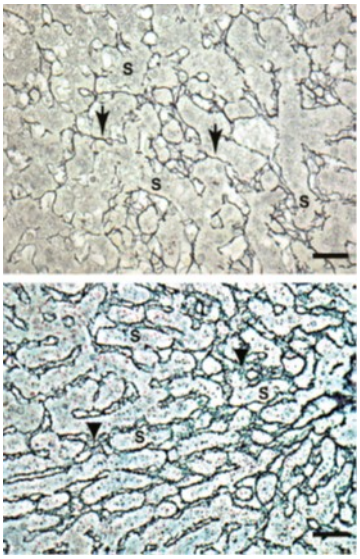

Figure 1 | Liver

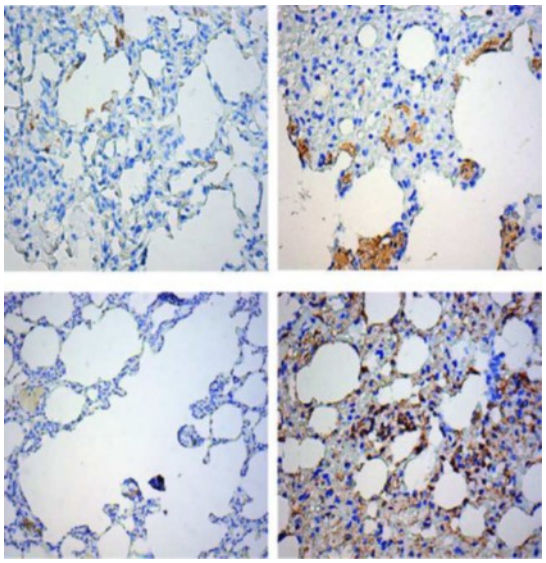

Figure 2 | Lung

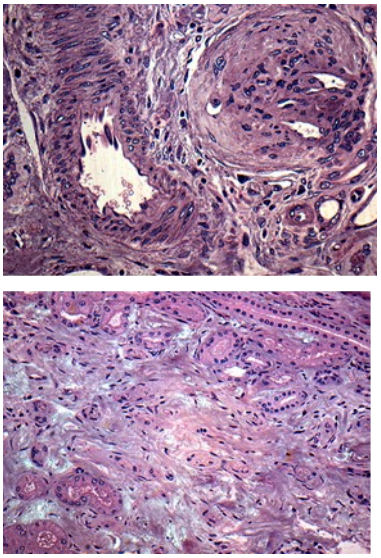

Figure 3 | Kidney

Figure 1 | (a-b): Liver sections of uninfected and infected dogs naturally infected with L. (L.) infantum: (a) Control dog – note a delicate network (weak colour black staining) of intralobular hepatic reticular fibres (arrows). (b) Symptomatic dog – note conspicuous collagen thickening in the space of Disse (stronger black staining of intralobular reticular fibres) (arrows). Some reticular fibres are coiled (arrowhead). Gomori's ammoniacal silver staining (bar = 32 μm). (S) Sinusoid blood vessel.

Figure 2 | Lungs sections of a naturally infected with Leishmania (L) infantum: (A) Lower magnification showing some areas with alpha smooth muscle actin (α-SMA) positive staining in brown evident the parenchyma (Bar = 32 μm). (B) High magnification showing alfa-actin (α-SMA) positive cells (pneumocytes and subepithelial layer) (Bar = 16 μm). (C-D) Note the expression of vimentin in low (C) and high magnification (D). It was always diffuse and localized mainly in the lung parenchyma cells, but always discrete in pneumocytes, unlike of α-SMA. (Bars = 32 and 16 μm, respectively). Immuno-streptavidin-peroxidase method.

Figure 3 | Kidney tissue sections showing in (A) sclerotic glomerular sclerosis (B) note a diffuse chronic tubulointerstitial fibrosis

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

The study of interaction between Leishmania and its host via evaluation of IL-8 and IL-23 genes expression

Ali akbar Shamsiyan [1*], Zahra Navi [1], Abdolrahim Rezaei [2], Narges Khazaei [1]

[1] Department of Parasitology and Mycology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran; [2] Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Iran.

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis is a protozoan infection which is mostly self- healing. Yet, in some cases lesions may spread because of lack of host resistant. The important immunologic indicators, involving the healing of CL in humans are not completely known. Cell-mediated immunity (CMI) plays an important role. The accessory cells and immune cells activity determine the healing or deterioration outcomes. For example, an appropriate increase in Th1 response against Leishmania infection is protective, while an increase in Th2 may worsen the signs and symptoms of disease [1]. In this study IL-8 and IL-23 genes expression in infected people by *Leishmania major were compared with no infected people*. Samples were prepared from 9 healthy (Leishmaniasis, HIV, HTLV1, HBV negative) individuals and 25 CL patients. 10 cc vein blood was taken, Peripheral blood mononuclear cells (PBMCs) were isolated and RNA was extracted and reverse transcribed to cDNA, Then Real time PCR was conducted and level of IL-8 and IL-23expression were measured by, Taq Man method before and after PHA stimulation of PBMCs. The results were statistically analyzed using SPSS v 16. The findings of this study indicated that the mean IL-8 gene expression in patients with major leishmaniasis is significantly increased compared with healthy samples, and this increase is significant (Mann-Whitney test, P = 0.038). Also, the mean gene expression of IL-23 in patients with major Leishmaniasis is higher than healthy subjects, but this increase is not significant. Comparison of the IL-8 gene expression level with the location of the lesion by Wilcoxon test showed that the IL-8 gene expression level was correlated with the location of the lesions. Particularly, patients who had face and hand ulcers indicated the most increase of IL8 level. At the end we can conclude that IL8 secretion which is correlated with neutrophil recruitment could be account for the first defense line of acquired and innate immunity in leishmaniasis. In addition, face and hand lesions are more stimulating and provoke immune system more effective. In comparison with IL8, IL23 is less important and shows slight difference among uninfected and infected individuals.

Acknowledgments:
We express our sincere thanks to Mashhad University of Medical Sciences, Iran for providing financial support for this study. This article was supported by Mashhad University of Medical Sciences, Iran (Project grants: 930781).

References:
1] Anderson CF, Stumhofer JS, Hunter CA, Sacks D, J Immunol 183 (2009) 4619-4627. doi:10.4049/jimmunol.0804024

Correspondence: Email - shamsiyana@mums.ac.ir

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Impact of miltefosine resistance and miltefosine treatment on virulence of *Leishmania infantum*

Eline Eberhardt [1], Dimitri Bulté [1], Lieselotte Van Bockstal [1], Sarah Hendrickx [1], Louis Maes [1], Guy Caljon [1]

[1] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium.

Available Online: 30 December 2018

ABSTRACT

Miltefosine (MIL) is the only oral drug for visceral leishmaniasis and although deficiency in an aminophospholipid/miltefosine transporter is sufficient to elicit drug resistance, only very few naturally MIL-resistant (MIL-R) parasites have yet been isolated. To study the impact of MIL resistance acquisition, two isogenic bioluminescent lines of *Leishmania infantum* that originated from a French HIV patient (MHOM/FR/96/LEM3323) were selected for differential susceptibility to MIL (MIL-S and MIL-R) [1], followed by transfection to express red-shifted luciferase (PpyRE9) which is a proficient tool for downstream *in vivo* bioluminescent imaging.

Experimental induction of MIL resistance, which was linked to a frameshift mutation in the MIL transporter [2], resulted in a reduced *in vitro* promastigote growth rate and a reduced metacyclogenesis. MIL-R parasites were still capable of infecting macrophages *in vitro* but displayed a decreased intracellular multiplication and a severely compromised virulence in mice. The MIL-S parental line produced maximal bioluminescent signals in liver, spleen and bone-marrow corresponding to a typical visceral infection progression, whereas MIL-R only showed a limited bioluminescent signal in the liver that disappeared by 3-4 wpi. This compromised dissemination of the MIL-R strain was associated to a high induction of pro-inflammatory cytokines such as IFN-g, IL-6 and TNF-a during the early onset of infection. Co-infection studies illustrated that MIL-S can outcompete MIL-R *in vivo* and episomal reconstitution with the wildtype MIL transporter was able to restore parasite virulence. Surprisingly, *in vivo* MIL-treatment or *in vitro* MIL-pre-exposure significantly rescued MIL-R parasite virulence in all target organs. *In vitro* exposure of MIL-R promastigote cultures to MIL modified the parasite morphology into a longer more slender shape, suggesting an altered parasite membrane composition.

Collectively, this experimental study demonstrates a detrimental impact of a non-functional MIL transporter on virulence/infectivity in mice. MIL-treatment of these parasites partially reverses this fitness loss and enables *in vivo* growth. These observations emphasize the importance of MIL-resistance profiling prior to drug administration.

Acknowledgments:
This work was funded by the Research Fund Flanders and a research fund of the University of Antwerp. LMPH is a partner of the Antwerp Drug Discovery Network (ADDN, www.addn.be) and the Excellence Centre 'Infla-Med' (www.uantwerpen.be/infla-med).

References:
1] Hendrickx S et al., Parasitology Research 115 (2015) 10 4061-4070.
2] Mondelaers A et al., PLoS One 11 (2016).

Correspondence: Email - guy.caljon@uantwerpen.be

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

In vitro drug susceptibility assessment of clinical isolates: which cell type to use?

Sarah Hendrickx [1], Guy Caljon [1], Louis Maes [1]

[1] Laboratory of Microbiology, Parasitology and Hygiene (LMPH), University of Antwerp, Antwerp, Belgium.

Available Online: 30 December 2018

ABSTRACT

Monitoring drug susceptibility of clinical *Leishmania* isolates still relies on standard in vitro drug-susceptibility assays. Although such assay is extensively used in laboratories all over the world, no standardized protocol is yet available resulting in the use of different parasite stages, a wide variety of host cells (both primary cells and cell lines), associated manipulations, diverse drug exposure times, detection methods and endpoint criteria. In the past, pleas have already been made for standardization to decrease inter-laboratory variation and to enable a more direct comparison of results [1]. Starting from the *in vitro* standard susceptibility assay on intracellular amastigotes, agreement should be reached at least on which host cell to use.

The advantages and disadvantages of using a number of primary cell types and cell lines were compared by screening their initial receptivity to four strains of *Leishmania infantum* promastigotes and their role to support intracellular multiplication. Additionally, the impact of primary cell stimulation and cell adherence time was explored for mouse primary peritoneal macrophages. An overall assessment of the cell's accessibility, manipulability and manageability was made, aiming to advocate one particular cell type that supports the most cheap, easy, rapid and straightforward drug susceptibility procedure.

Despite their proven differences in infection susceptibility and support in vivo, primary cells isolated from Swiss, BALB/c and C57BL/6 mice only showed minor alterations in terms of receptivity to infection, dependent on different cell stimulation procedures and cell adherence times. In addition, a few dissimilarities were noted between the different cell types with regard to support of intracellular multiplication.

The more practicable (e.g. cheapest) and suitable in vitro assay to measure drug susceptibility of clinical isolates involves primary peritoneal macrophages derived from Swiss mice obtained after 24h stimulation with starch and allowed to establish in culture for 48 hours before infection. Incubation takes 120h without change of the culture medium and overall parasite burdens are assessed microscopically upon Giemsa staining. In situations where primary cells are not accessible, THP1 cells represent the most pragmatic choice.

Acknowledgments:
This work was funded by the Research Fund Flanders and a research fund of the University of Antwerp. LMPH is a partner of the Antwerp Drug Discovery Network (ADDN, www.addn.be) and the Excellence Centre 'Infla-Med' (www.uantwerpen.be/infla-med).

References:
1] Hendrickx S, Guerin PJ, Caljon G, Croft SL, Maes L., Parasitology. 21 (2016) 1-11. doi:10.1017/S0031182016002031

Correspondence: Email - louis.maes@uantwerpen.be

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

New World cutaneous leishmaniasis in a traveller to a country endemic for Old World cutaneous leishmaniasis

C M Vassalos [1*], G Spanakos [2], A Vakali [2], A Mpimpa [3], N Tegos [3], E Vassalou [3]

[1] Greek Health System/Technological Institute of Athens; [2] Hellenic Centre for Disease Control and Prevention; [3] Department of Parasitology, Entomology and Tropical Diseases, National School of Public Health

Available Online: 30 December 2018

ABSTRACT

Cutaneous leishmaniasis (CL) is caused by both Old World (OW) and New World (NW) *Leishmania* species. We present an uncommon case of NWCL in a US tourist to Greece that is endemic for OWCL [1]. A 23-year-old female traveller to Greece was referred for laboratory evaluation of a non-healing, painless, 1.3-cm crusted ulcerative lesion on the left leg. She had presented to a local physician and received topical antibiotics. She recalled the first appearance of the lesion three weeks ago, while touring the Greek countryside. We removed the crust and scraped material from the lesion edge. Smear microscopic examination disclosed the presence of *Leishmania*, confirmed by a *Leishmania* genus-specific PCR. The PCR product was not cleaved by *Apo*I, thus excluding *L. infantum* as the causative agent, and indicating the possible presence of *L. tropica*, which is the most common cause of CL in Greece [2]. We asked our patient about her itinerary. She has been in the country only for two months in a period not corresponding to the seasonal activity period of sand flies in Greece, but she had been to Costa Rica two months before arriving in Greece. PCR product was subsequently sequenced. The sequence was identical to that of *L. panamensis* strain MHOM/PA/94/PSC-1. Therapy with mitelfosine was proposed, as NWCL may progress to mucocutaneous disease in contrast to often self-limited OWCL. Travellers with CL coming to a setting endemic for CL may be infected with *Leishmania* species that are common in their previous travel destinations. In these cases, molecular methods need to be employed as speciation is the key to choose an adequate treatment option as therapeutic recommendations differ according to species.

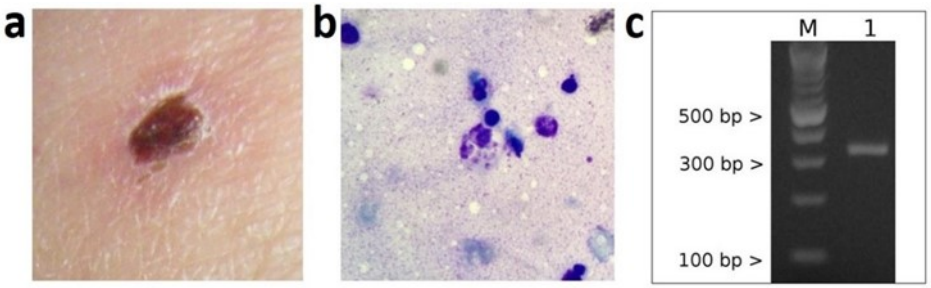

Figure 1 | a) Crusted ulcer on traveller's left leg. b). Amastigotes in macrophage. c) Lane 1: gel electrophoresis of the PCR product after digestion with the restriction endonuclease *Apo*I. M: Size marker (100 bp DNA ladder).

References:

1] J. Sratigos, A. Tosca, G. NIcolis, S. Papavasiliou, J. Capetanakis, International Journal of Dermatology 19 (1980) 2 86–88. doi:10.1111/j.1365-4362.1980.tb00257.x

2] G. Spanakos, E.-T. Piperaki, P.G. Menounos, N. Tegos, A. Flementakis, N.C. Vakalis, Transactions of the Royal Society of Tropical Medicine and Hygiene 102 (2008) 46–53. doi:10.1016/j.trstmh.2007.05.019

Correspondence: Email - vassalos.constantine@gmail.com

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

SPECIAL ISSUE: SELECTED ABSTRACTS OF THE I INTERNATIONAL CAPARICA CONFERENCE ON LEISHMANIASIS (LEISHMANIASIS 2018)

Transcriptome: identification of pathways involved in human pathogenesis of visceral Leishmaniasis.

Maykon Tavares de Oliveira [1*], Carlos Alessandro Fuzo [1], Sandra Regina Costa Maruyama [1], Allynne Karen Mendonça de Santana [1], José Marcos Ribeiro [1], Vanessa Carregaro [1], Amélia Ribeiro de Jesus [1], Roque Pacheco de Almeida [1], João Santana da Silva [1].

[1] Programa de Pós-graduação em Imunologia Básica e aplicada, Laboratório de Imunoparasitologia, Faculdade de Medicina de Ribeirão Preto – FMRP/USP, Universidade de São Paulo

Available Online: 30 December 2018

ABSTRACT

Leishmaniasis is a cluster of diseases caused by protozoa of the genus Leishmania and affects more than 12 million people worldwide, with 310 million people at risk of infection. In Brazil, L. infantum is the etiological agent of visceral leishmaniasis (VL), which presents a chronic, life-threatening, and widespread pathology. Among the infected individuals, 85% remain asymptomatic, while the remaining 15% present clinical manifestations, ranging from oligosymptomatic (mild) to more severe symptomatic forms, leading to death. We believe that the quality of the immune response generated by the host determines the final clinical outcome of the disease and that it would be directed by specific transcriptional profiles. Thus, our aim was characterize the peripheral blood transcriptomes of symptomatic infected individuals before and after conventional treatment, as well as of asymptomatic individuals, in order to obtain an overview of the genes involved in the disease pathogenesis. For this, the transcriptional profile was obtained through RNA-seq on the Illumina platform and analyzed by bioinformatics tools, which identified molecular processes involved in the immune response generation and / or modulation of different clinical outcomes of LV caused by L. infantum. This analysis enabled the discovery of ten pathways and several important gene targets, which may in future serve as therapeutic targets and biomarkers for the treatment and prognosis of Visceral Leishmaniasis.

Acknowledgments:
Thank the funding agencies for the financial support, FAPESP, CRID, FMRP-USP and CNPq.

References:
Queiroz, A., Sousa, R., Heine, C., Cardoso, M., Guimaraes, L.H., Machado, P.R., Carvalho, E.M., Riley, L.W., Wilson, M.E., Schriefer, A., J Clin Microbiol 50 (2012) 4028-4034. doi:10.1128/JCM.02064-12
Schriefer, A., Guimaraes, L.H., Machado, P.R., Lessa, M., Lessa, H.A., Lago, E., Ritt, G., Goes- Neto, A., Schriefer, A.L., Riley, L.W., Carvalho, E.M., Emerg Infect Dis 15 (2009) 871-876 doi:10.3201/eid1506.080406

Correspondence: Email - maykontavares@yahoo.com.br

Original Articles

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

ORIGINAL ARTICLE | DOI: 10.5584/jiomics.v8i4.221

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

Feiyu Yang [1]*, Yun Zou [1], Chunfang Ni [2], Chenggong Zhang [3], Fenjin, Sun [3], Xiaoliang Yuan [1], Rong Wang [2], Wenbin Liu [1]

[1] Shanghai Research Institute of Criminal Science and Technology, Shanghai Key Laboratory of Crime Scene Evidence, Shanghai, 200083 China; [2] Institute of Forensic Science, Shanghai Public Security Bureau,Shanghai Key Laboratory of Crime Scene Evidence, Shanghai 200083, China; [3] Shangai Police College, Shangai 200137, China.

Received: 29 November 2017 **Accepted:** 26 October 2018 **Available Online**: 7 November 2018

ABSTRACT

In this work, an automated magnetic dispersive solid-phase extraction (AMDSPE) sampling method followed by high performance liquid chromatography-mass spectrometry (HPLC-MS) was developed for quantitative enrichment of cocaine and its metabolites (COCs) from human urine, using modified magnetic nanoparticles as absorbents. The methodology was validated according to internationally accepted criteria in order to establish the validity of the combination of the AMDSPE and use of HPLC-MS for quantitative analysis. The proposed device significantly improved the sampling preparation efficiency with 32 samples in one batch within 40 mins. 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 and elution solvent. The automated procedure exhibited acceptable linearity ($r>0.9972$) over the concentration range of 10 to 200ng/mL. The limits of detection for the cocaine and its metabolites were 0.23-1.5 $ng{\cdot}mL^{-1}$ with recoveries ranging from 77.1 to 94.7%. Compared to traditional sampling method, this method is time-saving and environmentally friendly.

Keywords: Magnetic dispersive solid-phase extraction, Magnetic nanoparticles, Automation, Drug detection, High performance liquid chromatography.

1. Introduction

Computer Cocaine (COC) is the third most common substance of abuse after cannabis and alcohol. The use of cocaine as an illicit substance is implicated as a causative factor for multisystem derangements ranging from an acute crisis to chronic complications [1]. Growing consumption trend of abused COC and drug crimes are also a great concern, therefore, it is necessary to devote to find a simple, rapid and efficient methods for detection of cocaine and its metabolites (COCs) in human body in criminal technical field [2-7].

Urine drug testing is a noninvasive sampling whereas drugs and metabolites are usually present in high concentrations and relatively long detection windows [8]. However, direct analysis of urine samples is not feasible because urine complex medium often causes low sensitivity and selectivity of the determination [6,8]. Several manual pretreatment methods have been used for the extraction and clean-up of drugs or pesticide residue in complex matrices, such as liquid -liquid extraction [9], dispersive liquid–liquid microextraction [10-11], solid-phase extraction (SPE) [12-14], solid-phase microextraction and dispersive solid-phase extraction [15]. However, these techniques have many disadvantages such as tedious operation, long extraction time and large consumption of eluents. Especially when the amount is large, the pretreatment step would be more tedious and time-consuming. Nowadays, multi-analyte screening

***Corresponding author:** Dr. Fejyu Yang; e-mail: yangfyhit@sina.com. Shanghai Research Institute of Criminal Science and Technology, Shanghai Key Laboratory of Crime Scene Evidence, Shanghai, 200083 China.

methods can benefit from the development of the automated sample clean-up based on SPE to overcome the drawbacks of liquid-liquid extraction, while it still need to excitation and rinse steps. So, automation is very important in practical application of sample pretreatment, which can perform the extraction and solvent desorption steps of multiple samples in parallel and presents a time-efficient method.

In this work, a new automated method which can reduce the manual pretreatment steps and the risk of human error was developed. This kind of automated magnetic dispersive solid-phase extraction (AMDSPE) sampling method followed by high performance liquid chromatography-mass spectrometry (HPLC-MS) was researched for quantitative enrichment of COCs from human urine, using modified magnetic nanoparticles as absorbents. The nanoparticles were prepared by silanizing magnetic Fe_3O_4 nanoparticles and modifying them with divinyl benzene and vinyl pyrrolidone, which possesses the ability for specific adsorption of COCs. And this kind of magnetic particle facilitated the pretreatment steps by electromagnetically controlled extraction to achieve full automation.

2. Material and Methods

2.1 Chemicals and materials

COCs and its metabolites with 1.0 mg·mL^{-1} of Benzoylecgonine (BE), 1.0 mg·mL^{-1} of Norcocaine (NC), 1.0 mg·mL^{-1} of Ecgonine (ECG), 1.0 mg·mL^{-1} of m-Hydroxybenzoylecgonine (m-HOBE), 1.0 mg·mL^{-1} of Benzoylnorecgonine (BN), 1.0 mg·mL^{-1} of Norcocaethylene (NCE), 1.0 mg·mL^{-1} of Cocaine (COC), 1.0 mg·mL^{-1} of Cocaethylene (CE) and the internal standards 1.0 mg·mL^{-1} of BE-d^3, 100 μg·mL^{-1} of NC-d^3 hydrochloride were obtained from the National Institute for food and drug control (Beijing, China) and Cerilliant Corporation (Darmstadt, Germany). The methyl alcohol, acetone, acetonitrile (ACM), ethyl acetate, ethylene glycol, sodium acetate, ferric chloride, dimethylformamide, tetraethyl orthosilicate (TEOS), divinyl benzene (DVB), vinyl pyrrolidone (VP), isopropanol, 2,2-azobisisobutyronitrile, methacrylic acid-3-(trimethoxysilyl) propyl ester (MPS) were purchased from Beijing Chemicals Corporation (Beijing, China). The syringe filters were purchased from Xingya (Shanghai). All other chemicals were used as received without further purification.

The magnetic Fe_3O_4 particles were prepared according to our previous reported synthetic process [16]. Firstly, the silyl reagents MPS and TEOS were coated on the surface of Fe_3O_4 by hydrolysis in order to form SiO_2 layer. 1g as synthesized Fe_3O_4 was dispersed in 200 mL ethanol under ultrasonication, then 50mL water was added to the above dispersion with ultrasonication for 5 min. After stirred for 30min, 2mL ammonia was added to the solution. The temperature was set to 45°C, kept it for 20 minutes, then 4.5mL TEOS and 1.5mL MPS was added and stirred for another 12 h to obtain the SiO_2 layer. Secondly, 2 g as prepared particles were dispersed in 500 mL acetonitrile in the 1000 mL stand-up round bottom flask, then DVB (3.6 mL), VP (7.2 mL) and 2,2-azobisisobutyronitrile (0.4 g) were added to the solution. The mixture was allowed to react with vigorous stirring for 1 h with N_2. Then, the temperature was set to 75°C and this mixture was allowed to react for another 8h. Later the isolated material was washed 3 times with water and 3 times with ethanol. Finally, the product was dried at room temperature.

Scanning electron micrographs (SEM) were obtained with a S3400N scanning electron microscope (Hitachi, Japan). Infrared spectra were recorded by a Nicolet 6700 FT-IR spectrophotometer (Nicolet, USA). The magnetic properties were analyzed through a vibrating sample magnetometer (VSM, PPMS-9), which was purchased from Quantum Design, Ltd., USA.

2.2 Chromatography conditions and mass spectrometric conditions

Chromatographic separation was performed on an Agilent HPLC 1200 system with a C18 column (Agilent Eclipse XDB C18, 4.5 mm × 150 mm, 5μm) equipped with a guard column (Agilent Eclipse XDB C18, 4.5 mm × 12.5 mm, 5 μm), a G1311A quaternary pump, a G1329A autosampler, and a G1316A column oven. The mobile phase was composed of solvent A (2 mM ammonium formate and 0.05% formic acid in 96% water and 4% acetonitrile) and solvent B (2 mM ammonium formate and 0.05% formic acid in acetonitrile). The column was maintained at 45°C and eluted with a gradient of 10% B (0-1 min), 10-30% B (1-2 min), 30-50% B (2-6 min), 50-70% B (6-13 min), and 70-95% B (13-13.5 min), and the column was then flushed with 95% B (13.5–16.5 min), 95–10% B (16.5–18 min). The total run–time was 18 min at a flow rate of 0.20 mL/min. The temperature of the auto–sampler prior to analysis was maintained at 8°C. The injection volume was fixed at 5 μL in the partial loop with needle overfill mode. Mass spectrometry was performed on AB SCIEX API 4000 linear ion QTRAP quadrupole mass spectrometer (USA) equipped with an electrospray ionization (ESI) interface in the positive ion mode. The tandem mass spectrometer was operated under the multiple reaction monitoring mode, Q1 and Q3. Diluted stock solutions of each analyte and the internal standards were prepared in order to obtain the appropriate multiple reaction monitoring mode parameters. The optimal parameters were as follows: ion spray voltage was 5500 V, entrance potential was 10 V, collision cell exit potential was 10 V, curtain gas flow was 30 psi, Nebulizer gas and heating gas pressures (GS1 and GS2) were 50 and 60 psi respectively, collisional activated dissociation gas setting was medium, and source temperature was set at 600°C. Cone voltage (CV) was optimized to get the maximum intensity of the protonated molecular species $[M+H]^+$. The specific parameters for each analyte are shown in Table 1.

2.3 AMDSPE procedure for high-throughput analysis

Table 1. Optimum mass spectrometry (MS) conditions used for determination of COCs.

Compound	$[M+H]^+$ (m/z)	Retention time (min)	Collision Energy(eV)	Quantitation (m/z)	Scan time (s)
BE	290.3	7.77	45	105.1	0.3
NC	290.3	7.74	23	168.3	0.3
ECG	186.3	2.60	25	168.3	0.3
m-HOBE	306.1	7.58	29	168.1	0.3
BN	276.1	7.70	23	154.3	0.3
CE	318.0	8.15	30	196.1	0.3
NCE	304.1	7.98	35	136.2	0.3
COC	304.3	7.99	28	182.3	0.3
BE-d^3	293.4	7.72	42	105.3	0.3
NC-d^3	293.4	8.11	35	136.4	0.3

The sample preparation equipment with 96 holes location and touch display screen is shown in Fig.1a. Software was designed to fill the dialog boxes with the extraction process parameters directly. The sample plate with magnetic particles (green color), urine (yellow) and eluent (red) was shown with different colors in Fig1b. The AMDSPE procedure is shown in Fig.1c. The whole automatic pretreated procedures included the magnetic dispersion, solid phase extraction and elution steps. Firstly, the magnetic nanoparticles (50mg/ml) with 400 μL water solution was added into the tubes, and the particles were stirred up and down in water for about 1 min. Then the magnetic nanoparticles were moved and dispersed into the sample solution (urine or spiked solution) for extraction for 20 min with stirring rod up and down mixing. After the extraction step was completed, the particles were translated into desorption tube for eluting the target with stirring rod up and down mixing for 15 min by 1 ml desorption solvent (methanol: acetonitrile (3:7, v/v)). The tube containing the desorption solvent was then transferred into the HPLC-MS by syringe filter for analysis. The magnetic particles can be collected and reused after cleaning with acetone and 70% ethanol solution.

2.4 Method validation

The stock solutions of NCE, CE and NC were diluted with acetonitrile to 0.1 mg·mL^{-1}, and those of COC, BE, ECG, BN and m-HOBE were diluted with methanol to 0.1 mg·mL-1. The stock solutions of BE-d^3 and NC-d^3 were prepared in acetonitrile at 0.1mg·mL-1. The mixed working solutions for urine samples containing NCE, CE, NC, COC, BE, ECG, BN and m-HOBE at 0.1 and 1 μg·mL^{-1} were prepared in methanol. The internal standard working solutions for urine samples containing BE-d^3 and NC-d^3 were prepared in methanol at 0.1 μg·mL^{-1}. All solutions were stored at 4°C. To produce the desired concentrations for validation of each experiment and internal standardization, further dilutions in water were prepared on the same day.

A method validation covering all aspects (selectivity, linearity, accuracy, precision, matrix interferences, recovery, carry over and stability) required to establish the feasibility of a validated AMDSPE approach for analysis of the Cocaine and its metabolites was performed according regulatory guidelines. The working standard mixture solution at a concentration of 10 μg·mL^{-1} was prepared by appropriate dilution of the stock standard solutions with methanol. These solutions were stored at 4°C in the dark. Spiked recoveries for method precision and accuracy and matrix effects were performed at concentrations of 50 ng·mL^{-1} for COCs in urine samples. The spiked samples were homogenized in a tube and stored at 4°C for about 24 h. The method was evaluated by linearity, LOD and LOQ, precision and accuracy. Calibration standards in acetonitrile with concentrations 20.0, 25.0, 50.0, 100.0, 150.0 and 200.0 ng·mL^{-1} were prepared for the calibration curves. The limit of detection (LOD) and the limit of quantification (LOQ) were determined based on a signal-to-noise ratio of 3 (S/N=3) and 10 (S/N=10), respectively.

3. Results and discussion

3.1 Characterization of magnetic particles

The magnetic Fe_3O_4 particles were prepared according to our previous reported synthetic process [16]. The core-shell microstructure of the magnetic particles was shown in Fig.2. Fig. 2a was core particle of Fe_3O_4 with the diameter of 500 nm, and Fig.2b was the particle coated with SiO_2 medium layer with slippy surface, and Fig 2c showed the final functional particles with outer coating layer of divinyl benzene and vinyl pyrrolidone.

FT-IR spectra of the magnetic particles were shown in Fig.3. The peaks at 580 cm^{-1} and 1092 cm^{-1} are attributed to the stretching vibrations of Fe-O (Fig3a) and Si-O (Fig3b) respectively. In comparison to the curve of Fig3c, the characteristic peaks of C-H were found at approximately 1280 cm^{-1}. The strong absorption at 1518 cm^{-1} indicated the presence of C-O group in particle surface. All peaks showed that the synthesis was achieved and the ideal groups were obtained for sorbents.

Fig. 4 shows the hysteresis curve of magnetic particles at room temperature. As can be seen, the three curves have a similar shape and symmetry about the origin. The saturation magnetization value was found to be 69.3 emu·g^{-1} for Fe_3O_4 and 59.2 emu·g^{-1} for Fe_3O_4 with medium layer. This difference might be attributed to the non-magnetic SiO_2 shell surrounding the magnetite particles. After the outer layer was grafted on the particles, saturation magnetization value was 36.1 emu·g^{-1}. This indicated the new outer layer was formed.

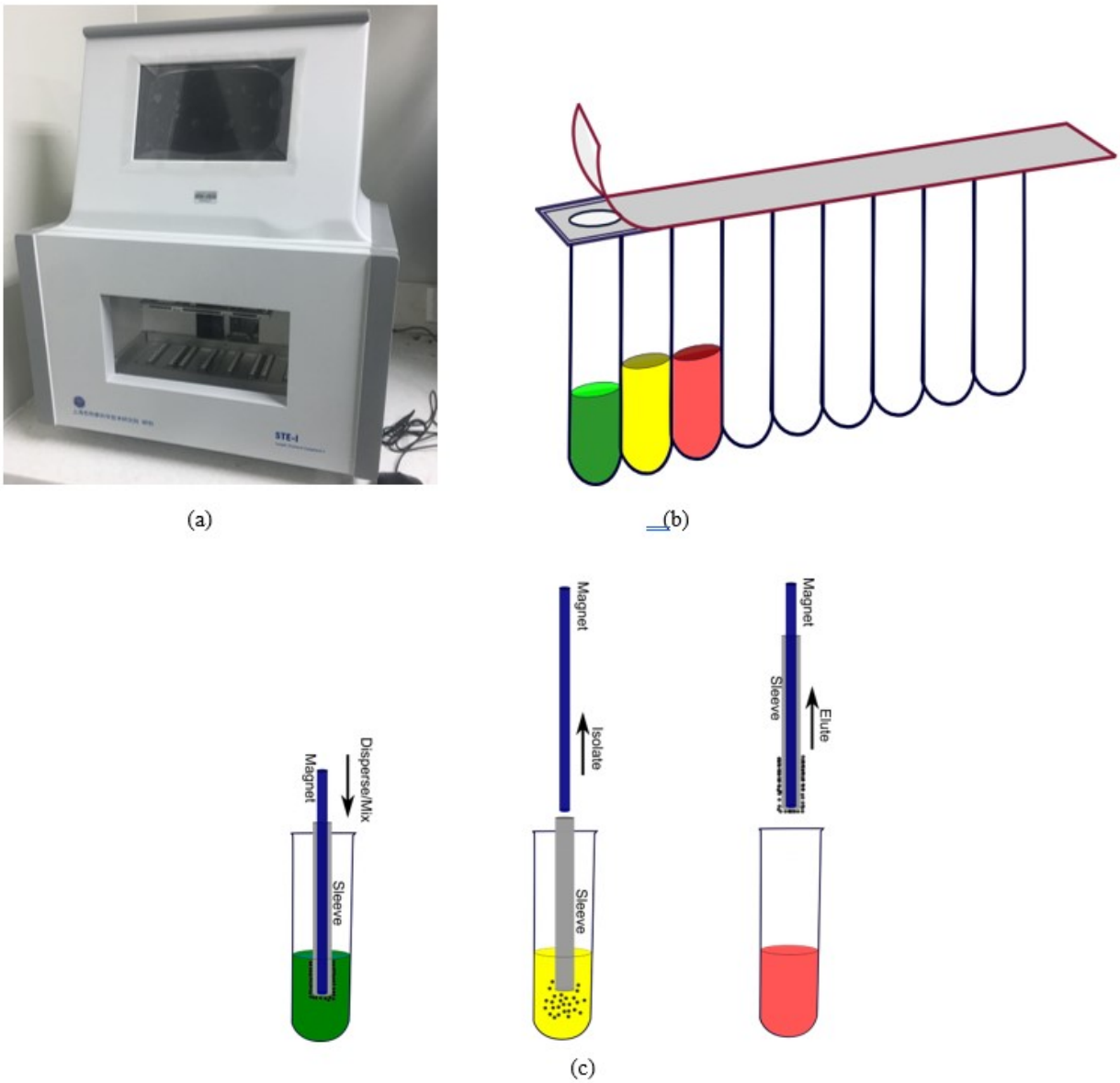

Figure 1. Automated sampling equipment(a), sample plate(b) and process of magnetic dispersive solid-phase extraction(c).

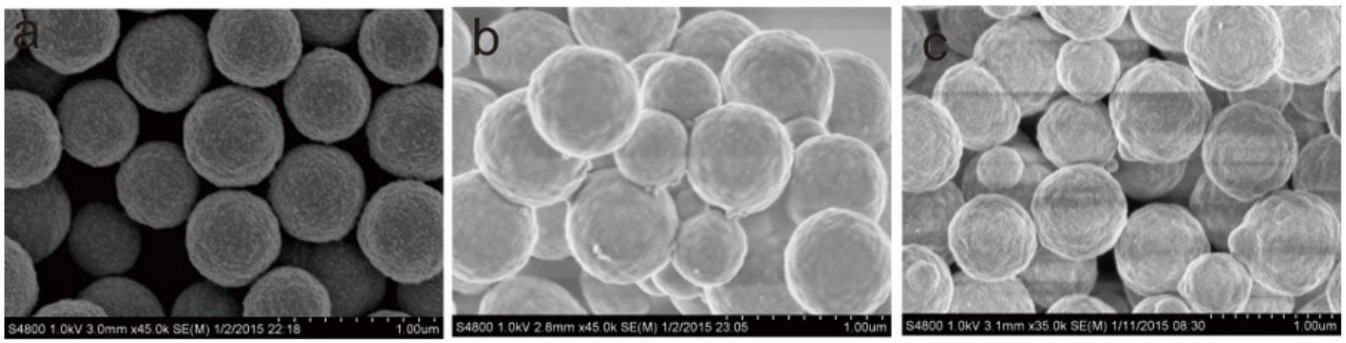

Figure 2. The SEM of (a) Fe3O4, (b) Fe3O4 with medium layerand (c) Fe3O4 with outer layer.

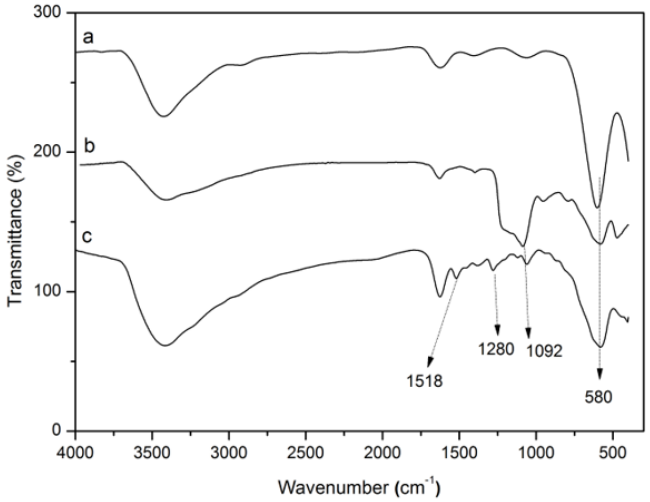

Figure 3. The Fourier transform infrared spectroscopy results of (a) Fe_3O_4, (b) Fe_3O_4 with medium layerand (c) Fe_3O_4 with outer layer.

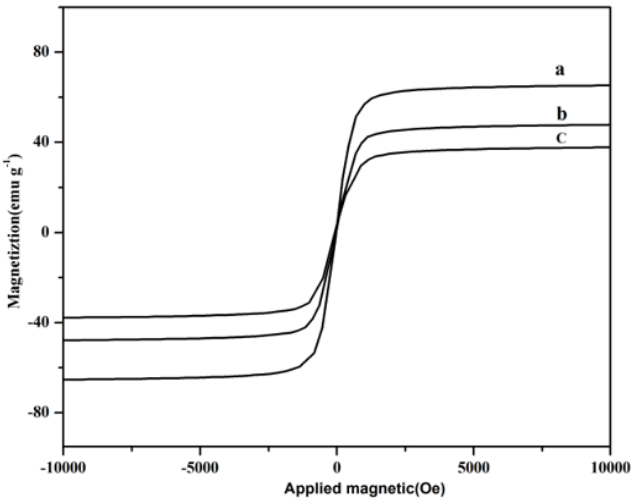

Figure 4. The magnetic property of (a) Fe_3O_4, (b) Fe_3O_4 with medium layerand (c) Fe_3O_4 with outer layer investigated by vibrating sample magnetometer.

3.2 Optimization of automated magnetic dispersive solid-phase extraction conditions

It is of importance to employ a suitable amount of the magnetic particles without affecting the recoveries of COCs.To investigate the optimum amount of adsorbents needed for the preconcentraion and extraction of COCs, batch experiments were performed by 1 mL of urine sample being spiked at 50.0 ng·mL-1 of COCs with the amount of the adsorbents from 1 to 50 mg. The results showed that the extraction efficiency increased with increasing amount of adsorbent up to 20 mg and then levelled off . The recoveries of the 8 COCs were in the range of 73.5- 94.7%. Thus, 20 mg of adsorbent was used for further experiment.

The type and the volume of the elution solvent are necessary parameters in elution procedure to obtain reliable results. To select the best eluent for desorbing analytes from the adsorbent, acetone, methanol, acetonitrile, ethyl acetate, water and their mixture solution were examined. The best elution of analytes was mixture solution (methanol: acetonitrile (3:7, v/v)), which ensure efficient and robust elute COCs while maintaining satisfactory recoveries of the target; it was selected as the elution solvent in the subsequent experiments. Furthermore, considering the minimum usage of organic solvents for reducing environment pollution, the volume of the elution solvent should be as small as possible in the desorption step. The effect of desorbing solvent volume on the recovery of COCs was investigated in the range of 0.1 to 3 mL. The maximum recoveries in the range of 78.4-99.8% were obtained with 1 mL. Therefore, 1 mL of elution solvent (methanol: acetonitrile (3:7, v/v)) was selected for the next experiments.

The time needed for the interaction between the adsorbate and the adsorbent is crucial. Therefore, the effect of adsorption time on the recoveries was studied. Batch experiments were performed by mixing 20 mg of magnetic particles in 1 mL of urine sample spiked at 50 ng·mL^{-1} with adsorption time in the range of 2 to 30 min. The experimental results indicated that the recoveries of eight COCs gradually increased and reached to an equilibrium of 78.2–101.3% with the increasing of the adsorption time from 2 to 20 min. Furthermore, the similar results were also obtained in the case of low concentration level of 20.0 ng·mL^{-1}. Therefore, 20 min was chosen as the optimal adsorption time for further studies.

3.3 Method validation

For the analysis of urine samples, the linearity of calibration curves made by peak area (y) vs. concentration (x, ng·mL^{-1}) was studied using calibration standards in freshly prepared urine samples at six concentrations of 10.0, 25.0, 50.0, 100.0, 150.0 and 200.0 ng·mL^{-1}. The response function was found to be linear. For eight kinds of COCs, the correlation coefficients were higher than 0.9972 in the tested range listed in Table 2. The LOD and LOQ, which were calculated on the analysis of 8 COCs in blank extracts spiked at low level in blank samples that yielded a signal-to-noise (S/N) ration of 3 and 10, were in the range of 0.23-1.5 ng·mL^{-1} and 0.69-4.7 ng·mL^{-1}, respectively. The stability, accuracy and precision were assessed based on the analysis of COCs spiked at 50 ng·mL^{-1} in blank urine sample. Table 2 shows that the majority of mean recoveries are in the range of 77.1–94.7% at the spiking levels, wherein the associated intra-day relative standard deviations (RSDs) vary from 3.2 to 6.8% and the inter-day (RSDs) vary from 2.1 to 6.2%.

To further verify the feasibility of this method, three batches of urine sample (32 samples for each batch) were analyzed by the developed method. Each batch of samples was processed together with a matrix blank (COCs-free sample), which was confirmed by using HPLC-MS method. The blank matrix was used to eliminate the false positive in the extraction process and instrument. The 8 COCs were identified by comparison of their retention time and

Table 2. Linear ranges, correlation coefficient(r), LOD, LOQ, intra-day/inter-day variation, recovery and RSD for COCs studied .

Analytes	Internal Standard	Linear range ng·mL^{-1}	r	LOD (ng·mL^{-1})	LOQ (ng·mL^{-1})	Intra-day/ Inter-day variation (%)	Recovery (%) 50ng·mL^{-1} (%RSD)
BE	BE-d^3	10-200	0.9981	0.23	0.79	4.1/6.2	81.3 (3.2)
NC	NC-d^3	10-200	0.9992	0.31	0.98	4.6/3.4	87.1 (4.5)
ECG	BE-d^3	10-200	0.9972	1.5	4.7	3.2/5.5	94.7(5.2)
m-HOBE	BE-d^3	10-200	0.9982	0.23	0.69	6.3/2.1	82.3 (3.8)
BN	BE-d^3	10-200	0.9977	0.36	1.11	3.5/5.2	77.1 (4.2)
CE	NC-d^3	10-200	0.9972	0.46	1.58	3.3/2.7	81.3 (5.6)
NCE	NC-d^3	10-200	0.9983	0.41	1.45	4.8/4.2	79.3 (3.5)
COC	NC-d^3	10-200	0.9982	0.59	2.02	6.8/4.8	87.5 (4.9)

fragment ions with the related standard compound. No COCs were detected in the 32 real urine samples in first batch. Only one sample in third batch was detected containing ECG, and the typical positive sample HPLC-MS chromatogram is shown in Fig. 5.

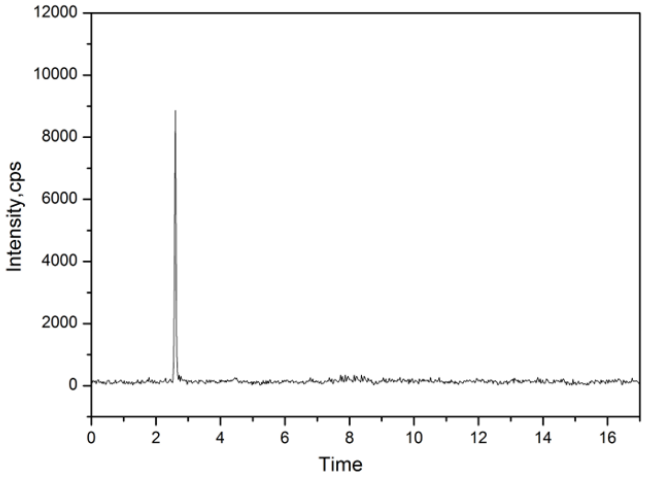

Figure 5. The positive sample HPLC-MS chromatogram with ECG detected.

4. Conclusion

In this study, the special magnetic material was prepared for selectively adsorb the COCs by electromagnetic immobilization and enrichment, rendering the sample preparation easier and controllable. The automated sample preparation equipment provided reproducilbe and reliable results, and allowed for high throughput simultaneous analysis of up to 32 samples, which corresponds to approximately 1.2 min per sample, while manual manipulation is 60 min for about six samples. The proposed method was validated by samples spiked with analytes mixture of satisfactory recovery, repeatability and efficiency, which help to fulfill a simple, fast and automated way for determination COCs in human urine. In quantitive analysis and high-throughput cases, this newly designed method will provide an effective tool for direct extraction of target analytes in complex mixtures with low matrix interference and limit of detection. Meanwhile, the present work provides a promising application for the analysis of other persistent drugs or toxicant in complex biological sample.

Acknowledgements

This research was supported by the Technology Research Projects of Ministry of Public Security of China (Grant No.2015JSYJB06), the Project of National Natural Science Foundation of China (Grant No. 61405117) and the Project of National Natural Science Foundation of China (Grant No.81701864) and shanghai Natural Science Foundation of China (Grant No. 17ZR1441600).

References

1] Xiong, L. J., Wang, R., Liang, C., Cao, F. Q., Rao, Y. L., Wang, X., Zeng, L. B., Ni, C. F., Ye, H. Y., Zhang, Y. R., Determination of ecgonine and seven other cocaine metabolites in human urine and whole blood by ultra-high-pressure liquid chromatography–quadrupole time-of-flight mass spectrometry. Anal. Bioanal. Chem. 2013, 405, 9805-9816.

2] Clauwaert, K. M., Van-Bocxlaer, J. F., Lambert, W. E., De-Leenheer, A. P., Segmental analysis for cocaine and metabolites by HPLC in hair of suspected drug overdose cases. Forensic Sci. Int. 2000, 110, 157-166.

3] Fernandez, P., Vazquez, C., Morales, L., Bermejo, A. M., Analysis of opiates, cocaine and metabolites in urine by high-performance liquid chromatography with diode array detection (HPLC-DAD). J. Appl. Toxicol. 2005, 25, 200-204.

4] Jeanville, P. M., Estape, E. S., Torres-Negron, I., Marti, A., Rapid confirmation/quantitation of ecgonine methyl ester, benzoylecgonine, and cocaine in urine using on-line extraction coupled with fast HPLC and tandem mass spectrometry. J. Anal. Toxicol. 2001, 25, 69-75.

5] Shakleya, D. M., Huestis, M. A., Simultaneous quantification of nicotine, opioids, cocaine, and metabolites in human fetal postmortem brain by liquid chromatography tandem mass spectrometry. Anal. Bioanal. Chem. 2009, 393, 1957-1965.

6] Gareri, J., Klein, J., Koren, G., Drugs of abuse testing in meconium. Clinica Chimica Acta. 2006,366, 101-111.

7] Paulsen, R. B., Wilkins, D. G., Slawson, M. H., Shaw, K., Rollins, D. E., Effect of four laboratory decontamination procedures on the quantitative determination of cocaine and metabolites in hair by HPLC-MS. J. Anal. Toxicol. 2001, 25, 490-496.

8] Esteveromero J., Albiolchiva J., Perisvicente J., A review on development of analytical methods to determine monitorable drugs in serum and urine by micellar liquid chromatography using direct injection. Anal Chim. Acta. 2016, 926, 1-16.

9] Zhao, X. N., Liu, X. J., Zhao, Z. X., Huang, C. J., Zhang, M. H., Wang, H. L., Wang, X. D., Homogeneous liquid–liquid extraction combined with high performance liquid chromatography–fluorescence detection for determination of polycyclic aromatic hydrocarbons in vegetables. J. Sep. Sci. 2009, 32, 2051-2057.

10] Farajzadeh, M. A., Seyedi, S. E., Shalamzari, M. S., Bamorowat, M., Dispersive liquid–liquid microextraction using extraction solvent lighter than water. J. Sep. Sci. 2009, 32, 3191-3200.

11] Zhao, R. S., Diao, C. P., Chen, Q. F., Wang, X., Sensitive determination of amide herbicides in environmental water samples by a combination of solid-phase extraction and dispersive liquid–liquid microextraction prior to GC–MS. J. Sep. Sci. 2009, 32, 1069-1074.

12] Jiang, F. L., Rao, Y. L., Wang, R., Johansen, S. S., Ni, C. F., Liang, C., Zheng, S. Q., Ye, H. Y., Zhang, Y. R., Sensitive, automatic method for the determination of diazepam and its five metabolites in human oral fluid by online solid-phase extraction and liquid chromatography with tandem mass spectrometry. J. Sep. Sci. 2016, 39, 1873-1883.

13] Liu, X. Y., Zheng, S. Q., Jiang, Z., Liang, C., Wang, R., Zhou, Z., Zhang, Y. R., Yu, Y. Q., Rapid separation and identification of Strychnos alkaloids metabolites in rats by ultra high performance liquid chromatography with linear ion trap Orbitrap mass spectrometry. J. Sep. Sci. 2014, 37, 764-774.

14] Teng, X. M., Liang, C., Wang, R., Sun, T., Rao, Y. L., Ni, C. F., Zeng, L. B., Xiong, L. J., Li, Y., Zhang, Y. R., Screening of drugs of abuse and toxic compounds in human whole blood using online solid-phase extraction and high-performance liquid chromatography with time-of-flight mass spectrometry. J. Sep. Sci. 2015, 38, 50-59.

15] Snow, N. H., Solid-phase micro-extraction of drugs from biological matrices. J. Chromatogr. A 2000, 885, 445-455.

16] YangF.Y., Zou Y., Ni C.F. ,Wang R., Wu M., Liang C., Zhang J.B., Yuan X.L., Liu W.B., Magnetic dispersive solid-phase extraction based modified magnetic nanoparticles for the detection of cocaine and cocaine metabolites in human urine by HPLC-MS, J. Sep. Sci..DOI: 10.1002/jssc.201700457.

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

ORIGINAL ARTICLE | DOI: 10.5584/jiomics.v8i4.230

Recovering Damaged Documents to Improve Information Retrieval Processes

Ángel Luis Garrido[1*], Alvaro Peiró[2]

[1] SID Research Group, University of Zaragoza, Pedro Cerbuna 12, 50009 Zaragoza, Spain ; [2] InSynergy Consulting S.A., Calle de López de Hoyos141, planta 2º - 28002 Madrid, Spain.

Received: 31 December 2017 **Accepted:** 22 October 2018 **Available Online**: 19 December 2018

ABSTRACT

Although computer forensics is frequently related to the investigation of computer crimes, it can also be used in civil procedures. An example of case of use is information retrieval from damaged documents, where words have undergone alterations, either accidentally or intentionally. In this paper, we present a new tool able to retrieve information from large volumes of documents whose contents have been damaged. We have designed a new approach to recover the original words, composed of two stages: a text cleaning filter, able to remove non relevant information, and a text correction unit, which gather a general purpose spell checker with a N-gram based spell checker built specifically for the domain of the documents. The benefits of using this combined approach are two-fold: on the one hand, the general spell checker allows us to leverage all the general purpose techniques that are usually used to perform the corrections; on the other hand, the use of an N-gram based model allows us to adapt them to the particular domain we are tackling exploiting text regularities detected in successfully processed domain documents. The result of the correction allows us to improve automatic information retrieval tasks of from the texts. We have tested it using a real data set by using an information extraction tool based on semantic technologies in collaboration with the Spanish company InSynergy Consulting.

Keywords: Information Recovery, Text Cleaning, Spell Check, N-gram.

1. Introduction

Computer forensics is a specialty within forensic science disciplines. The goal of computer forensics is the search for evidence in digital data, involving the preservation, identification, storage media extraction, documentation, and interpretation [1]. Computer forensics can also be used in civil procedures, and information recovery is one of these scenarios. In fact, correcting texts, also known as "text cleaning", is a well-known task in the field Natural Language Processing [2] and it has been widely used in many related works [3]. We can find different cases of use for the application of text cleaning inside the field of computer forensics, especially regarding activities related to the reconstruction of documents for legal, police, administrative or industrial purposes. In this work, we propose a text cleaning system for automatically recovering data from large documents, whose functioning is composed of two main steps: 1) a Text Cleaning Filter, able to remove non relevant information, as headings, page numbers, stamps, etc., and 2) a Text Correction Unit, which combines a general purpose spell checker with a N-gram based spell checker built specifically for the domain of the documents we are dealing with. The main contribution of our approach is the experimental study of how the application of N-gram together with the grammar checker can contribute to the

***Corresponding author:** Dr. Ángel Luis Garrido; e-mail: garrido@unizar.es. SID Research Group. Department of Computer Science and Systems Engineering. School of Engineering and Architecture. Ed. Ada Byron, Maria de Luna, 1, 50018, Zaragoza (Spain) Phone: +34 654 833 616. E-mail address: garrido@unizar.es.

improvement of a text cleaning task. For that, we have integrated our implementation in AIS [6,7], an information extraction system within the domain of legal texts, where extraction process is guided by a specific domain ontology for the typology of the document.

2. Material and Methods

In real-world scenarios, systems must deal many times with documents which are actually scanned images. In such cases, to extract the text contents out of the documents, a broadly adopted solution is to apply an Optical Character Recognition (OCR) system over the documents before any information retrieval task. Its effectiveness clearly influences the quality of the results, but even when the OCR is perfect, spelling problems or noisy words can be found. So, we propose a two-stage system with the aim of improving the text quality before a hypothetical data retrieval process:

1) After the OCR process, the first stage for the potentially damaged documents consists of removing non relevant information from the input to get the text as clean as possible for the correction step. The list of non-relevant items controlled by our system is as follows: document headers and foots, page numbers, stamps, spots, noisy characters, and signatures. All them difficult the retrieval process. These elements are removed by using regular expressions on the text.

2) The second stage is in charge of correcting the misspelled words which appear in the text. For this purpose, we advocate to apply a general purpose spell checker and, whenever it is possible, to enrich it with an N-gram based spell checker built specifically for the domain of the documents we are dealing with. We have used Hunspell [10], a powerful spell checker and morphological analyzer which offers a good multi-language support (e.g., English, French, Spanish, etc.), and we have developed our own N-gram libraries. For each detected error for a given word (wi), 1) we get the spell checker suggestions (si), and, 2) we assign each of them a score based on an adaptation of the Needleman-Wunsch [8] distance and computed with the actual word, and we reinforce them with the probabilities of the N-grams suggestions as follows:

$$wordScore(wi, si) = gSP(wi, si) * NG(wi)^{\alpha} \quad (1)$$

where gSP is the score based on the metric distance, and NG is the probability of wi being the next word in the domain where it appears. Both values are normalized in the 0..1 range. The α is used to assign a weight to the NG(wi) value in order to give more or less relevance to the suggested word.

Note that our system only accepts words that are suggested by the spell checker, and gets their probability from the N-gram suggestion list. Besides, wordScore is never 0 as gSP(wi) always returns a value greater than 0, as we add perplexity to our N-gram model using the add-one Laplace Smoothing method [9].

3. Results

For the experiments, we have used a dataset formed by 250 notarial purchase documents in Spanish. These documents have been selected randomly from the private repository of InSynergy Consulting (ISYC), a company devoted to document management. These 250 documents have between 100 and 200 pages and each document was manually revised by expert professionals in the field to detect text errors. We have trained our domain N-gram model using 7,500 already refined notarial documents in Spanish, also available in the private repository of ISYC. After testing with a 5-Gram model, we finally have used in the experiments a 3-Gram model because of its better behavior, comprising 1,213,920 different sequences and a vocabulary of 174,831 different words. As we wanted to evaluate how our domain N-gram influenced the quality of the text correction on the ISYC dataset, we carried out a set of experiments in order to: 1) evaluate if a domain N-gram contributes to the performance of text correction in the presence of a general spell checker, and 2) select the best combination of Hunspell and the domain N-gram model by varying the α parameter.

4. Discussion

In Table 1, we show the obtained results, where α = 0 corresponds to the case where the system only considers the information provided by Hunspell. We analyzed the results

Table 1. Results obtained using Hunspell and domain 3-Gram over ISYC dataset. For Hits, greater is better; for Misses and FP, lower is better, and for FN, greater is worse.

	α value						
	0	**0.01**	**0.02**	**0.03 - 0.09**	**0.1**	**0.2**	**0.3**
HITS	617	623 ▲0.97%	632 ▲1.46%	**634** **▲1.78%**	634 ▲1.78%	630 ▲1.13%	630 ▲1.13%
MISSES	816	810 ▼0.86%	806 ▼1.23%	**798** **▼1.47%**	802 ▼0.98%	802 ▼0.98%	802 ▼0.98%
FP	366	288 ▼13.11%	288 ▼13.11%	**288** **▼13.11%**	288 ▼13.11%	288 ▼13.11%	288 ▼13.11%
FN	239	240 ▲0.42%	240 ▲0.42%	**240** **▲0.42%**	240 ▲0.42%	240 ▲0.42%	240 ▲0.42%

considering the typical matrix of confusion: Hits (the system correction is equal to the real word), Misses (the system correction is different to the real word), False Positives (FP) (the system corrects a word that was already correct), and False Negatives (FN) (the system does not correct a word that was incorrect). We can see that the optimal value of α in Equation 1 according to our test data must be in the range (0.03 - 0.1), and the results are stabilized from 0.1 and onwards. Regarding misses, FP and FN, they are mainly due to Hunspell failures.

5. Concluding Remarks

In this paper, we have described the design of a text cleaning system for correcting errors of damaged scanned documents. Also, we have enhanced the correction step by using an N-gram model that weights all the suggestions made by a well-known spell checker. The word sequences which frequently appear in a particular document typology make our system to be able to perform a highly adapted word-level correction for that kind of documents. As a future work, we want to improve the correction process by adding new methods and tools, for example, with the incorporation of a different spell-check, a fine grained machine learning system and natural language processing techniques like rule based grammars in the correction step. We also plan to test the behavior in other scenarios different from the scope of legal documents, since the initial requirements are the same.

Acknowledgements

This research work has been supported by projects TIN2013-46238-C4-4-R, TIN2016-78011-C4-3-R (AEI/FEDER, UE), and DGA/FEDER. We want to thank ISYC, Dr. Carlos Bobed, and María G. Buey for their collaboration in this work.

References

1] W. G. Kruse II, J. G. Heiser, Computer forensics: incident response essentials. Pearson Education (2001). ISBN 0-201-70719-5.

2] C.D. Manning, H. Schütze, Foundations of statistical natural language processing. MIT Press (1999). doi: 10.1017/S1351324902212851

3] C. Biemann, A. Mehler, Text Mining: From Ontology Learning to Automated Text Processing Applications. Springer (2014) ISBN 978-3-319-12654-8. doi: 10.1007/978-3-319-12655-5

4] M. G. Buey, A.L., Garrido, C. Bobed, S. Ilarri, The AIS Project: Boosting Information Extraction from Legal Documents by using Ontologies. Proceedings of the 8th International Conference on Agents and Artificial Intelligence (2016) 438-445. doi: 10.5220/0005757204380445

5] M. G. Buey, C. Roman, A. L. Garrido, C. Bobed, and E. Mena, Automatic Legal Document Analysis: Improving the Results of Information Extraction Processes using an Ontology. Studies of Big Data, Springer (to be published in 2018)

6] S. B. Needleman, and C. D. Wunsch. A general method applicable to the search for similarities in the amino acid sequence of two proteins. Journal of molecular biology, 48(3) (1970) 443-453. doi: 10.1016/0022-2836(70)90057-4

7] J. H. Martin, and D. Jurafsky. Speech and language processing. International Edition, (2000).710. ISBN 978-0-13-187321-6

8] http://hunspell.github.io/ Last accessed: December 2017.

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

ORIGINAL ARTICLE | DOI: 10.5584/jiomics.v8i4.236

The Forensic Application Of Proteomics For The Study Of The Time Of Death: An Operative Experimental Model For PMI Estimation

Isabella Aquila[1]*, Sacco M.A.[1], Gratteri S.[2], Raffaele R.[1], Ricci P.[1,2]

[1] Institute of Legal Medicine, University "Magna Graecia" of Catanzaro. Italy; [2] Institute Institute of Legal Medicine, University of Foggia. Italy.

Received: 17 December 2017 **Accepted:** 24 September 2018 **Available Online**: 22 October 2018

ABSTRACT

Post Mortem Interval (PMI) estimation is one of the most important questions the forensic pathologist must answer. To date, it is not possible to establish exactly the hour of death, but only to calculate a period (PMI), during which death most likely occurred. In the forensic field, several laboratory methods can be used to perform this calculation more accurately. However, there is still no biomarker that is universally validated and accepted by the forensic community. In the literature, researches about the application of proteomics for forensic purposes are on the increase. 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 from patients who died at the Intensive Care Unit (AO "Mater Domini" of Catanzaro). The proposed operating model has several advantages including the evaluation, for the first time, of human biological samples from the exact moment of death. The analysis would allow to identify new potential biomarkers expressed in peripheral blood and validate the forensic application of markers already known in the literature. The knowledge of the exact moment of death (time 0) would allow us to evaluate the proteomic profile more accurately on the human model, overstepping the limits of some extrinsic variables evidenced in the literature.

Keywords: Forensic sciences, Proteomics, Time of death, Post Mortem Interval, Biomarker, Protein.

Abbreviations: PMI (Post Mortem Interval).

1. Introduction

Time of death is one of the greatest forensic enigmas. The evaluation of the time of death is performed by the forensic pathologist through the Post Mortem Interval (PMI) estimation, i.e. a time range in which death likely happened. This estimate is based on the calculation of a time interval that is formulated by studying the abiotic and transformative processes occurred on the corpse [1-2]. For the calculation of the PMI, supportive tests such as laboratory analysis on biological fluids, like the measurement of K + concentration in the vitreous humor, can also be used. To date, however, there is no biomarker that has been universally validated by the forensic community for the diagnosis of the time of death. Various scientific studies have been carried out for this purpose. Among them, many methods were analyzed in the literature, focusing on the post-mortem modifications of DNA, microRNA and protein modifications. Specifically, the number of studies proposed on protein analysis for PMI estimation is steadily increasing and have shown promising results.

Proteomics is the discipline that takes care of studying proteins from a systematic point of view. It can provide information about the structure, function, protein-protein interactions and quantitative variations with different

***Corresponding author:** Dr. Isabella Aquila; e-mail: isabella.aquila@hotmail.it. Legal Medicine Institute, University Magna Graecia of Catanzaro; Viale Europa, Loc. Germaneto, 88100 Catanzaro, Italy. Telephone: 0039-09613647304. Fax: 0039-09613647560. Email: isabella.aquila@hotmail.it.

methods. Among them there are: ELISA assay, Western Blotting, 2D PAGE (Two Dimensional Polyacrylamide gel Electrophoresis) method used in combination with mass spectrometry and LC-MS/MS (Liquid chromatography-tandem mass spectrometry) strategies .

As part of the study of PMI, multiple proteins expressed in various animal and human tissues, such as skeletal muscle, heart and brain, have been analyzed. The scientific evidences published so far in the literature revealed the potential of some proteins as possible biomarkers related to the time elapsed since death [3-5].

The great limitations associated with proteomic investigations so far have to do with the exposure of the corpse to the outside temperature, as it is an extrinsic variable that can alter the kinetics of protein degradation but, above all, the lack of proteomic analysis on humans from the exact time of the death. Indeed, this evaluation has so far been carried out only on tissues and organs of animals and never on humans. This is due to the obvious difficulty of knowing the exact time of death on human (time 0), the inability to obtain in humans an estimation of proteins expressed from the exact time of death and the difficulty of analyzing the corpse for a long-time interval. The exact knowledge of the time of death would allow us to accurately estimate the variation of one or more proteins without the interference of some variables, such as the time elapsed before the corpse's finding and the exposure temperature.

In this paper, we want to propose an operating model, still being applied and analyzed, using human blood as biological sample. This protocol is estimated to be easy to reproduce and it could be used as a reference model for post-mortem proteomic analysis on humans. This protocol, applied on humans, would greatly contribute to the studies of the relationship of one or more proteins with PMI.

2. Material and Methods

The proposed operating model was accepted by the Ethical Committee of the A.O. Mater Domini of Catanzaro. The procedure involved taking peripheral venous blood samples from deceased patients at the U.O. of Anesthesia and Resuscitation of the University Hospital of Catanzaro. The samples were taken at the U.O. of Anesthesia, so the exposure temperature of the corpses was the Room Temperature (around 25° C). The blood draws were only performed after the signed informed consent of the patient's family members. Specifically, venous blood drawings from cephalic vein were carried out on patients who have cardiac arrest and then die. From the exact time of death (time 0), blood samples were collected at predetermined time intervals, up to 2 hours after death. This time interval was chosen because corpses can be held by law in the Department of Anesthesia only up to 2 hours after death. Furthermore, the primary aim of the work was to evaluate the so-called "early post mortem interval". The samples were immediately centrifuged (2500 rpm for 5 minutes) and the plasma was extracted and stored in appropriate tubes (2 ml centrifuge tubes) at -80 °. Samples were subjected to proteomic analysis by ELISA immunoenzymatic method ongoing. In our project, the levels of the following proteins will be measured:

- HMGB-1 (High Mobility Group Box 1);
- Cardiac Troponin T..

There are also other candidates:

- Cardiac Troponin I;
- GFAP (Glial fibrillary acidic protein);
- Talin.

24 cases were collected in total. The analyzed cases were broken down by age, sex, comorbidities, and cause of death.

3. Results

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 post mortem interval investigated, but also qualitative ones. According to the available scientific evidences 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, that showed time dependent variations such as:

1. Ubiquitous cellular proteins, like HMGB1 (High Mobility Group Box 1): in serum samples, this protein has already proved to progressively increase with respect to time [6];

2. Specific organ proteins:

   - Muscle proteins due to progressive degradation, such as cTn I (Cardiac Troponin-I) and cTnT (Cardiac Troponin-T) [7-8];

   - Proteins of the brain tissue, such as GFAP (Glial fibrillary acidic protein) or talin, respectively with an increase and a reduction [9-10].

This is the first work that aims to measure the levels of these proteins in human plasma after death. To date, only HMGB-1 has been evaluated on animal serum [6]. These proteins are not typical plasma proteins. This data could have some benefits and some limitations. The benefit is that

the finding of these proteins in the plasma after death is not influenced by pre-existing plasma levels of the same proteins. The limitation is that the proteins could be not detectable in human plasma.

4. Discussion

In the literature, it has been shown that several proteins may undergo quantitative variations after death. Some of them exhibit a linear or pseudolinear kinetics of reduction due to the progressive degradation of the protein [8]; others of them show a post-mortem increase [6]. Several proteins expressed in both ubiquitous and specific tissues, whether animal or human, have already been evaluated [3-5].

For example, Kikuchi et al. have shown that the HMGB-1 protein progressively increases after death, using the mouse blood as analytical sample. It is a ubiquitous and abundant protein in the cell nucleus. ELISA assay showed that this protein may also increase up to seven days after death, with temperature-dependent kinetics variations [6]. These results have not yet been validated on a human model.

In the cardiac tissue, it has been shown that both the degraded cTnI intact protein and the degraded cTnT percentage decrease progressively analyzed after death, proportionally to the elapsed time interval. These analyzes were carried out, in the first case on an animal and human model, in the second case on a human model [7-9].

Concerning the brain tissue, interesting results regarding GFAP and talin proteins have been obtained. GFAP is a protein that has shown to increase in human samples of substantia nigra; talin has shown to diminish in the human brains analyzed after death. Both analysis were performed using Western Blotting [10-11].

All the results described and obtained on humans point the limitations related to some variables, such as the temperature, the cause of the death, but above all the time elapsed since death before the analysis was performed.

No research has thus far been conducted to analyze the variation of one or more proteins on the human model from the exact moment of death. For this purpose, we have proposed an experimental operating model under analysis that tries to overcome many of the limitations described.

Indeed, this protocol provided for the taking of peripheral venous blood drains on the corpse knowing the exact time of death (time 0), from patients who had a cardiac arrest and subsequently died. The pros of the analysis of the cardiac arrest group is that we were certain of the exact moment when the cardiac activity stopped. The possible cons is that cardiac arrest could have influence on the levels of some markers that are related to the cardiac damage, such as Troponin T. The analyzed cases were divided by age, sex, comorbidities, and cause of death in order to standardize the data obtained and to better estimate the influence of extrinsic variables on proteic variation in time.

The samples were taken at predetermined standard time intervals and with a constant exposure temperature of the corpse (Room Temperature). In addition, all the samples taken were subjected to the same procedure for analysis, i.e. immediate centrifugation, plasma extraction and subsequent storage at -80 ° C. In addition, the sample that has been selected for analysis (blood) is extremely easy to draw and can also be analyzed with other proteomic methods such as Western Blotting or Mass Spectrometry.

The next step in the protocol involves the performing of ELISA immunoassay for all the samples taken and is still being analyzed. The investigation would reveal the qualitative and quantitative variations of all selected proteins between the different post-mortem intervals. This would allow not only to confirm the data already available literature but also to discover new candidate proteins to become potential biomarkers of PMI.

5. Concluding Remarks

Our study has several advantages:

- Identify new potential biomarkers of time elapsed since death, expressed in human blood;
- 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;
- Know the kinetics of one or more proteins after death from time 0.

Acknowledgements

Preliminary analyses were accepted and presented at I International Caparica Conference in Translational Forensics and accepted for presentation at AAFS 70th Annual Scientific Meeting 2018 in Seattle.

References

1] I. Aquila, F. Pepe, C. Di Nunzio, F. Ausania, A. Serra, P. Ricci. J Forensic Sci. 59 (2014) 1665-1667. DOI: 10.1111/1556-4029.12538.

2] I. Aquila, S. Boca, F. Caputo, M.A. Sacco, S. Gratteri, V. Fineschi, P. Ricci. Am J Forensic Med Pathol. (2017) 229-232. DOI: 10.1097/PAF.0000000000000317.

3] S. Pittner, B. Ehrenfellner, A. Zissler, V. Racher, W. Trutschnig, A.C. Bathke, A.M. Sänger, W. Stoiber, P. Steinbacher, F.C. Monticelli. Int J Legal Med. 131 (2017) 479-483.

4] E.E. Foditsch, A.M. Saenger, F.C. Monticelli. Int J Legal Med. 130 (2016) 433-40. DOI: 10.1007/s00414-016-1459-4.

5] S. Pittner, B. Ehrenfellner, F.C. Monticelli, A. Zissler, A.M. Sänger, W. Stoiber, P. Steinbacher. Int J Legal Med. 130 (2016) 1547-1555. DOI: 10.1007/s00414-016-1349-9.

6] K. Kikuchi, K.I. Kawahara, K.K Biswas, T. Ito, S. Tancharoen, N. Shiomi, Y. Koda, F. Matsuda, Y. Morimoto, Y. Oyama, K. Takenouchi, N. Miura, N. Arimura, Y. Nawa, S. Arimura, M.X. Jie, B. Shrestha, M. Iwata, K. Mera, H. Sameshima, Y. Ohno, R. Maenosono, Y. Tajima, H. Uchikado, T. Kuramoto, K. Nakayama, M. Shigemori, Y. Yoshida, T. Hashiguchi, I.

Maruyama. Exp Ther Med. 1 (2010) 109-111. DOI: 10.3892/etm_00000019

7] A.J. Sabucedo, K.G. Furton. Forensic Sci Int. 134 (2003) 11-16. DOI: 10.1016/S0379-0738(03)00080-X.

8] S. Kumar, W. Ali, S. Bhattacharya, A.K. Verma. J Forensic Leg Med. 40 (2016) 16-21. DOI: 10.1016/j.jflm.2016.02.002.

9] S. Kumar, W. Ali, U.S. Singh, A. Kumar, S. Bhattacharya, A.K. Verma, R. Rupani. J Forensic Sci. 61 (2016) S241-2455. DOI: 10.1111/1556-4029.12928

10] A. Crecelius, A. Götz, T. Arzberger, T. Fröhlich, G.J. Arnold, I. Ferrer, H.A. Kretzschmar. Proteomics. 8 (2008) 1276-1291. DOI: 10.1002/pmic.200700728

11] Z.K. Campell, I. Kwon, S.J. Finley, Y. Lee, G.T. Javan. J Forensic Leg Med. 44 (2016) 188-191. DOI: 10.1016/j.jflm.2016.10.020