

**BIOGRAPHICAL SKETCH**

NAME: d'Aloja, Ernesto

eRA COMMONS USER NAME (credential, e.g., agency login): EDALOJA

POSITION TITLE: Full Professor of Legal Medicine

**EDUCATION/TRAINING**

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
Catholic University of Milan, Medicine Faculty of Rome, Italy	MD	10/1984	Medicine and Surgery
Catholic University of Milan, Medicine Faculty of Rome, Italy	PhD	7/1988	Forensic Sciences
Catholic University of Milan, Medicine Faculty of Rome, Italy	Residency	7/1991	Legal Medicine
Catholic University of Milan, Medicine Faculty of Rome, Italy	Fellow	9/1991	Forensic Sciences - Genetics

**A. Personal Statement**

The goal of the proposed research is to 1) employ pre-clinical models of vancomycin AKI to quantify the effects of concomitant nephrotoxins and ameliorating agents on vancomycin-exposure AKI profile in order to create Precision Dosing schemes for clinical application and 2) define critical time points and specific metabolomic profiles that identify kidney injury before meaningful functional changes occur. My contribution in the present project will be the identification of underlying mechanisms of kidney damage and/or protection starting from the urine and blood metabolomic profile modifications and proposing plausible biological correlations among identified metabolites, urinary biomarkers (eg KIM-1 and clusterin) and kidney histo-pathological features. These correlations will be investigated both in rat and in pig model. The alleged ameliorating action of one or more treatment will be tested against the identified modifications of urine and plasma metabolome of treated animals, even by ameliorating agent alone or in combination with vancomycin. I have over 30 years of experience in forensic pathology and more than 10 years involvement in investigating metabolomics responses to different *noxae*, either exogenous or endogenous, brought to living organisms. My scientific contribution may be demonstrated by several publications dealing with metabolomics investigation of some of the more intriguing bodily damaging mechanisms in humans. All these papers are focused on the understanding of the more relevant metabolites movements (increasing/decreasing) in body fluids as a consequence of an insult, so to imagine a protective treatment or a preventive approach, such as hypoxic-ischemic encephalopathy (HIE) in the newborn or asphyxial cardiac arrest (ACA) in adults. All these issues may have a direct/indirect interest in a legal medicine context as a frequent cause both of death and/or of malpractice lawsuit. Being one of the ultimate goals of the proposed study patient's safety, I believe my expertise will be of some improvement to all the other expertise so far involved in the project. I will cooperate with dr. Locci in the analysis of samples provided by Prof. Scheetz (rat model) and Prof. Xanthos (pig model), with whom I had a previous and sound scientific collaboration (see shared publications). I'm eager to be involved in the project.

1. Pais GM, Liu J, Avedissian SN, Hiner D, Xanthos T, Chalkias A, **d'Aloja E**, Locci E, Gilchrist A, Prozialeck WC, Rhodes NJ, Lodise TP, Fitzgerald JC, Downes KJ, Zuppa AF, Scheetz MH. Lack of synergistic nephrotoxicity between vancomycin and piperacillin/tazobactam in a rat model and a confirmatory cellular model. *J Antimicrob Chemother.* 2020;75(5):1228-1236. PubMed PMID: [32011685](#).
2. Varvarousis D, Xanthos T, Ferino G, Noto A, Iacovidou N, Mura M, Scano P, Chalkias A, Papalois A, De-Giorgio F, Baldi A, Mura P, Staikou C, Stocchero M, Finco G, **d'Aloja E**, Locci E. Metabolomics profiling reveals different patterns in an animal model of asphyxial and dysrhythmic cardiac arrest. *Sci Rep.* 2017;7:16575. PubMed PMID: [29185486](#). PMCID: [PMC5707403](#).

3. Locci E, Liu J, Pais GM, Chighine, A, Kahnamonei DA, Xanthos T, Chalkias A, Lee A, Hauser AR, Chang J, Rhodes NJ, **d'Aloja E**, Scheetz MH. Urinary Metabolomics from a Dose-Fractionated Polymyxin B Rat Model of Acute Kidney Injury. bioRxiv. 2021:444980. doi: 10.1101/2021.05.21.444980.
4. Chalkias A, Pavlopoulos F, Koutsovasilis A, **d'Aloja E**, Xanthos T. Airway pressure and outcome of out-of-hospital cardiac arrest: a prospective observational study. Resuscitation 2017 Jan;110:101-106. doi: 10.1016/j.resuscitation.2016.10.023. PubMed PMID: [27840003](https://pubmed.ncbi.nlm.nih.gov/27840003/).

### **Select Completed Research Support**

Ministero della Salute-CCM (Italian Health Ministry; 31/01/2014 – 31/12/2017) –

National competitive funding program

Project title: Benzene- and Polycyclic Aromatic Polycarbons (PAHs)- related carcinogenesis

Principal Investigator and National Coordinator: prof. Ernesto d'Aloja

The project was aimed at the collections on 'real world' data concerning the risk of exposure to Benzene and PAHs in the general population. For this reason an environmental data together with biological samples (urine and, in selected cases, blood) of citizens in different areas of Italy were prospectively collected.

A metabolomics analysis was performed and compared with 'traditional' clinical and analytical results.

The analysis was not able to show a better definition of biological risk in the general population achieved by metabolomics profiling.

Fondazione di Sardegna (Regional Funding Entity; 2018)

Regional competitive funding program

Project title : Blood-Spinal Cord Barrier (BSCB) and its involvement in post-aortic surgery spinal cord damage [Blood-Spinal Cord Barrier (BSCB) – Un modello di danno midollare] (Prot. U247.2018/AI.233.CN)

Principal Investigator and Regional Coordinator: prof. Ernesto d'Aloja

The main aim of the project was the investigation of biological mechanisms underlying the spinal cord damage after a surgical procedure (aortic prosthesis). in a longitudinal study of CerebroSpinal Fluid (CSF) samples.

### **B. Positions and Honors**

2010 to present – Director of the Residency Program in Legal Medicine, University of Cagliari

2005 to present – Full Professor, Legal Medicine Unit, University of Cagliari

1998 - 2005 - Associate Professor

1995 - 1998 - Assistant Professor

### **Other Experience and Professional Memberships**

1994 – 2000 – Italian Representative in the European DNA Profiling (EDNAP) Group

01/1995 to present – Member, International Society of Forensic Genetics (ISFG)

01/1995 to present – Member, Italian Group of Forensic Genetics (GeFI)

01/1995 to present – Member, Italian Group of Forensic Pathologist (GIPF)

01/1995 to present – Member, Italian Society of Legal Medicine (SIMLA)

01/1996 to present – Member, International Academy of Legal Medicine (IALM)

01/1996 - 04/1996 – Visiting Professor and International Scholar in Forensic Sciences University of South Alabama (USA) at Mobile and University of Alabama at Birmingham

2008 - 2014 – President of the Italian Group of Forensic Genetics (GEFI)

2012 - 2016 – Head of the Public Health Department at the University of Cagliari

2014 - 2017 – Past-President of the Italian Group of Forensic Genetics (GeFI)

2005 to present – Clinical Director of the Forensic Medicine Unit at the Cagliari Teaching Hospital

2005 to present – President of the Independent Ethical Committee at the Azienda Ospedaliera-Universitaria of Cagliari

### **C. Contributions to Science**

My contribution to science consists of 152 publications in high IF international journals (h-index of 25 with 2160 citations), in more than 100 papers published in Italian Journal of Legal Medicine, and in 14 book chapters. I started working in the forensic fields immediately after my medical degree, trying to promote the forensic laboratory innovation. My first interest was the mono (1D) e bi-dimensional (2D) electrophoretic separation of polymorphic serum proteins to be introduced in the forensic hematological lab for bloodstain identification and disputed paternity purposes and soon after I was involved in the implementation of DNA profiling methods in the

same field (starting from Multi-locus and Single-locus probes to PCR-amplified VNTR and STR till multiplexing of STRs from a single DNA extraction and mitochondrial haplotyping). In the 1994-2000 period I served as the Italian representative in the European DNA Profiling (EDNAP) Group, and the main achievement of the Group was standardization of EU profiling protocols and their harmonization with USA CODIS system. The interest in 'omics' sciences led me to start – together with dr. Locci and other colleagues – investigating the metabolome modification following several external and internal *noxæ* of public health or legal interest. In the last decade, we as a group achieved an in-depth knowledge and expertise in profiling several biofluids and tissues, collected both by living human subjects or by corpses which underwent autopsy and several animals (rat, sheep and goat, pig). As a forensic pathologist, my actual interest is the analysis of putative relationship between biofluids profiles and histopathological features of different causes of death of forensic cases interest. Relying my expertise on both fields (metabolomics and forensic pathology), the research interests are quite polymorphic, encompassing single- or multi-organ failure (with special attention devoted to heart and kidney), several 'possible' damaging agents (ranging from pathophysiological ones – such as hypoxia/anoxia, drugs of abuse, metals, stress-related manifestation, etc), and post-mortem time-related metabolomic modifications. In the view to define in the sharpest way the time since death, a peculiar efforts has been put forward in the last few years in the field of forensic ophthalmology. The publications listed below demonstrate my expertise in the standardized cooperative DNA profiling approach (1), in forensic pathology (2), in post-mortem ocular changes (3), in the use of metabolomics both in animal (4) and living human studies (5).

### **1. DNA profiling standardization and trans-national harmonization.**

Several papers were devoted to the efforts made by the EDNAP group to achieve a standardization of nomenclature, procedures, applicability, interpretation, and reporting of DNA profiles obtained by several biological sources. The main achievements of the collaborative exercises and of the creation of shared nomenclature, protocols, and inter-European allelic frequencies at a predetermined set of somatic loci together with haplotype frequencies of Y-related loci and mtDNA are nowadays the basis for the proper functioning of DNA databases of criminal offenders employed by several Countries in Europe and for the DNA information interchangeability among European Law Enforcement Agencies. As a cooperative group EDNAP set up a quality control and quality assurance (QCQA) program which is still in use at a European Community level.

a. Gill P, Kimpton C, **d'Aloja E**, Andersen JF, Bar W, Brinkmann B, Holgersson S, Johnsson V, Kloosterman AD, Lareu MV, et al. Report of the European DNA profiling group (EDNAP) – towards standardisation of Short tandem Repeat (STR) loci. *Forensic Sci Int.* 1994 Mar; 65(1):51-9. doi: 10.1016/0379-0738(94)90299-2. PubMed PMID: [8206453](#).

b. Gill P, Brinkmann B, **d'Aloja E**, Andersen J, Bar W, Carracedo A, Dupuy B, Eriksen B, Jangblad M, Johnsson V, Kloosterman AD, Lincoln P, Morling N, Rand S, Sabatier M, Scheithauer R, Schneider P, Vide MC, Considerations from the European DNA profiling group (EDNAP) concerning STR nomenclature. *Forensic Sci Int.* 1997 June 23; 87(3):185-92. doi: 10.1016/s0379-0738(97)00111-4. PubMed PMID: [9248038](#).

c. Carracedo A, **d'Aloja E**, Dupuy B, Jangblad A, Karjalainen M, Lambert C, Parson W, Pfeiffer H, Pfitzinger H, Sabatier M, Syndercombe Court D, Vide C. Reproducibility of mtDNA analysis between laboratories: a report of the European DNA profiling group (EDNAP). *Forensic Sci Int.* 1998 Nov 9;97(2-3):165-70. doi: 10.1016/s0379-0738(98)00154-6. PubMed PMID: [9871995](#).

d. Schneider PM, **d'Aloja E**, Dupuy BM, Eriksen B, Jangblad A, Kloosterman AD, Kratzer A, Lareu MV, Pfitzinger H, Rand S, Scheithauer R, Schmitter H, Skitsa I, Syndercombe-Court D, Vide MC. Result of collaborative study regarding the standardization of the Y-linked STR system DYS385 by the European DNA Profiling (EDNAP) group. *Forensic Sci Int.* 1999 Jun 28;102(2-3):159-165. doi: 10.1016/s0379-0738(99)00049-3. PubMed PMID: [10464931](#).

### **2. Forensic pathology.**

Several papers deal classic issues related to forensic pathology. A major attention of my actual scientific interest relies on the research of new and more objective, reliable, reproducible among laboratories, and less operator-dependent tools to make a sounder inference on time since death from one or more biological phenomena. In this perspective, one of the classical signs of death - *rigor mortis* - has been addressed by technologies employed in other clinical context (elasto-sonography used in neurology to assess spasticity in living patients) or in basic science (Atomic Force Microscopy, AFM, used to evaluate nano-mechanics). The genetic background derived from my experience in the field of personal identification and disputed paternity has been employed to explore the Sudden Cardiac Death (SCD) issue in an 'olistic approach' coupling gross anatomo-pathological findings, histopathological ones, toxicological, and genetic analysis.

a. De-Giorgio F, Nardini M, Foti F, Minelli E, Papi M, **d'Aloja E**, Pascali VL, De Spirito M, Ciasca G. A novel method for post-mortem interval estimation based on tissue nano-mechanics. *Int J Legal Med.* 2019 Jul;133(4):1133-1139. doi: 10.1007/s00414-019-02034-z. PubMed PMID: [30919038](#).

b. Demontis R, **d'Aloja E**, Manieli C, Carai A, Boi M, Serra MP, Quartu M. Case report of sudden death after a gunshot wound to the C2 vertebral bone without direct spinal cord injury: histopathological analysis of spinal-medullary junction. *Forensic Sci. Int.* 2019 Aug;301:e49-e54. doi: 10.1016/j.forsciint.2019.06.010. PubMed PMID: [31230858](#).

c. De Giorgio F, Grassi VM, Polacco M, Pascali VL, **d'Aloja E**, Arena V. Myocardial bridging and Sudden Cardiac Death: is the actual classification exhaustive? *Int J Cardiol.* 2014 Apr 1;172(3):e383-384. doi: 10.1016/j.ijcard.2013.12.286. PubMed PMID: [24508108](#).

d. Oliva A, Brugada R, **d'Aloja E**, Boschi I, Partemi S, Brugada J, Pascali VL. State of the art in forensic investigation of sudden cardiac death. *Am J Forensic Pathol.* 2011 Mar;32(1):1-12. doi: 10.1097/PAF.0b013e3181c2dc96. PubMed PMID: [20083991](#).

### **3. Post-mortem ocular changes.**

In the last few years the focus of my research group was to test the ability of Optical Coherence Tomography (OCT), a diagnostic tool in several ocular diseases (of both anterior and posterior segments of eye, together with the study of retina and vitreous humour) to describe in a qualitative (by a morphological approach) and in a quantitative (by serial pachymetric measurements of corneal tissues) way corneal post-mortem modifications. The major aim of this approach was to provide forensic community a composite tool to unravel the post-mortem interval issue by the description of abiotic modifications occurring in the ocular tissues from several different, but interconnected, points of view. Several papers have been published on an ovine model (in order to cover a wide, well defined, and useful timespan) giving a snapshot of biological modification of corneal tissues, aqueous humor, vitreous humor, and – only marginally – retina. Several experiments are actually ongoing to translate these preliminary results to human corpses and to validate them against the best gold standard for each time window (early, intermediate, and long PMI).

a. Napoli PE, Nioi M, **d'Aloja E**, Fossarello M. Post-mortem corneal thickness measurements with a Portable Optical Coherence Tomography System: a reliability study. *Sci Rep.* 2016 Jul 26;6:30428. doi: 10.1038/srep30428. PubMed PMID: [27457021](#)

b. Nioi M, Napoli PE, Demontis R, Locci E, Fossarello M, **d'Aloja E**. Morphological analysis of corneal findings modifications after death: a preliminary OCT study on an animal model. *Exp Eye Res.* 2018 Apr;169:20-27. doi: 10.1016/j.exer.2018.01.013. PubMed PMID: [29360448](#).

c. Napoli PE, Nioi M, **d'Aloja E**, Fossarello M. The Bull's eye pattern of the tear film in humans during fixation on En-face Optical Coherence Tomography. *Sci Rep.* 2019 Feb 5;1413. doi: 10.1038/s41598-018-38260-5. PubMed PMID: [30723239](#).

d. Nioi M, Napoli PE, Mayerson SM, Fossarello M, **d'Aloja E**. Optical Coherence Tomography in forensic science: a review of the literature. *Forensic Sci Med Pathol.* 2019 Sep;15(3):445-452. doi: 10.1007/s12024-019-013-z. PubMed PMID: [31321632](#).

### **4. Metabolomics in animal models.**

With the advent of 'omics' sciences, a new analytical tool has been implemented in the forensic arena: the metabolomics. Based on the expertise of dr. Locci, my group started to investigate classical forensic issues – such as post-mortem interval prediction, differential diagnosis of several common causes of death (sepsis, cardiac arrest, asphyxia), bodily effects of metals and drugs – by the use of <sup>1</sup>H NMR. One of the main drawback in forensic experiments is its peculiar context, related to post-mortem modifications due from one side to the cessation of blood flow and respiration – and as a consequence the progressive energetic derangement at several organ and cellular level the progressive failure of vital activities – and on the other the progressive and time-related change of microbial environment. If so, forensic scientists have to thoroughly identify in their results the findings from the injurious agent under investigation and separate them from the ones coming from the death processes. In our institute an experimental approach on animal models has been pursued whenever possible. In this view ovine model of PMI changes has been employed – knowing the exact moment of death and minimizing all the verifiable variables (such as age, sex, weight, diet, and genetic asset) – while in the studying of Hypoxia-Ischemia Encephalopathy (HIE) a piglet model has been investigated. Both approaches led to the description of a metabolomics profile both in aqueous humor (PMI in sheep) and in urine (HIE in piglets). Only

after the validation in the animal models, the identified metabolomics profile of interest may be tested with 'real world' specimens collected in the everyday forensic activity.

a. Locci E, Stocchero M, Noto A, Chighine A, Natali L, Napoli PE, Caria R, De-Giorgio F, Nioi M, **d'Aloja E**. A <sup>1</sup>H NMR metabolomic approach for the estimation of the time since death using aqueous humour: an animal model. *Metabolomics*. 2019 May 8;15(5):76. doi: 10.1007/s11306-019-1533-2. PubMed PMID [31069551](#).

b. Locci E, Scano P, Rosa MF, Nioi M, Noto A, Atzori L, Demontis R, De-Giorgio F, **d'Aloja E**. A metabolomic approach to animal vitreous humor topographical composition: a pilot study. *PLoS One*. 2014 May 20;9(5):e97773. doi: 10.1371/journal.pone.0097773. ecollection 2014. PubMed PMID: [24845217](#).

c. Fanos V, Noto A, Xanthos T, Lussu M, Murgia F, Barberini L, Finco G, **d'Aloja E**, Papalois A, Iacovidou N, Atzori L. Metabolomics network characterization of resuscitation after normocapnic hypoxia in a newborn piglet model supports the hypothesis that room air is better. *Biomed Res Int*. 2014;2014:731620. doi: 10.1155/2014/731620. PubMed PMID: [2496864](#).

d. Atzori L, Xanthos T, Barberini L, Antonucci R, Murgia F, Lussu M, Aroni F, Varsami M, Papalois A, Lai A, **d'Aloja E**, Iacovidou N, Fanos V. A metabolomic approach in an experimental model of hypoxia-reoxygenation in newborn piglets: urine predicts outcome. *J Matern Fetal Neonatal Med*. 2010 Oct;23 suppl 3:134-7. doi: 10.3109/14767058.2010.517033. PubMed PMID: [20873980](#).

### **5. Metabolomics in human livings and related issues.**

As a collateral interest of my research field, being legal medicine specialists in Italy involved in giving legal expertise on causal relationship between a variety of insults and a plaintiff's alleged corporal permanent damage or death my research group in the last years has been involved in collaboration with research groups focused on the understanding of potential mechanisms of damage. In this view the collaboration with prof. Xanthos led to the achievement of a better knowledge on basic mechanism underlying the cardiac arrest whenever the initial cause is asphyxia or ventricular fibrillation. The compelling need to transfer the metabolomics profiling results to the clinical scenario (from bench to bedside) has boosted the laboratory, experimental and clinical collaboration. We described a plasma metabolomics profile in the swine model able to discriminate between these two entities (ACA and VFCA) and ongoing research are focusing on its sensibility and specificity in a forensic context so to be translated to the clinics. In the field of public health, we recently described two urinary metabolomics profile related to the cardiac effects of shift-working and to the background exposure to arsenic in the drinkable water. In the first case (a case-control study), the urinary metabolomics profile was related to the stress to which workers who are obliged to do shift-working and to the prolongation of QTc, whenever compared with individuals exposed to the same working task but with two different timetables. In the other case, a selected population of wellbeing individuals was tested for a putative correlation between the increasing amount of 'background' arsenic – not coming from occupational exposure – and the urinary metabolomics profile. An intriguing correlation between the higher value of arsenic and metabolites characterizing a specific metabolomics profile has been depicted and these metabolites are the ones described in murine models of high concentration arsenic renal damage. This preliminary results seem to challenge the assumption - employed by policy makers and health authorities worldwide – that a 'safe threshold' value for arsenic content in drinkable water exists and that it may abolished or changed due to local situation.

a. Locci E, Lecca LI, Piras R, Noto A, Pilia I, **d'Aloja E**, Campagna M. Urinary <sup>1</sup>H NMR metabolomics profile of Italian citizens exposed to background levels of arsenic: a (pre)cautionary tale. *Biomarkers*. 2019 Dec;24(8):727-734. doi: 10.1080/1354750X.2019.1677777. Pubmed PMID: [31613149](#).

b. Chalkias A, Pavlopoulos F, Koutsovasilis A, **d'Aloja E**, Xanthos T. Airway pressure and outcome of out-of-hospital cardiac arrest: a prospective observational study. *Resuscitation*. 2017 Jan;110:101-106. doi:10.1016/j.resuscitation.2016.10.023. PubMed PMID: [278400](#).

c. Campagna M, Locci E, Piras R, Noto A, Lecca LI, Pilia I, Cocco P, **d'Aloja E**, Scano P. Metabolomic patterns associated to QTc interval in shiftworkers: an explorative analysis. *Biomarkers*. 2016 Nov;21(7):607-13. doi: 10.3109/1354750X.2016.1171900. PubMed PMID [27121294](#).

d. Varvarousis D, Varvarousi G, Iacovidou N, **d'Aloja E**, Gulati A, Xanthos T. The pathophysiologies of asphyxial vs dysrhythmic cardiac arrest: implications for resuscitation and post-event management. *Am J Emerg Med*. 2015 Sep;33(9):1297-304. doi: 10.1016/j.ajem.2015.06.066. PubMed PMID: [26233618](#).

**All the my publications may be found at:**

<https://www.ncbi.nlm.nih.gov/myncbi/ernesto.d'aloja.1/bibliography/public/>