Universitat de Girona

# I Jornada del programa de doctorat en Biologia Molecular, Biomedicina i Salut

## Abstract Book

Dra. Elisabeth Pinart (ed.)

Universitat de Girona


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## *CONTENTS*

# Origin and dispersal of the zebra mussel invasion in the Iberian Peninsula

**Luis Peñarrubia, Nuria Sanz, Jordi Viñas, Oriol Vidal**

Keywords: genetic diagnostic; invasive species; massive parallel sequencing; population genetics; zebra mussel.

## *Introduction*

The zebra mussel (*Dreissena polymorpha*, Pallas, 1771) is a bivalve native to the Ponto-Caspian region. It is considered one of the world's 100 worst invasive alien species, generating severe ecological and economic impacts. In the Iberian Peninsula, since the recent invasion in 2001, *D. polymorpha* has expanded along the Ebro River basin and colonized adjacent basins in the northeastern Iberian Peninsula.

Current methodologies for *D. polymorpha* detection are based on visual diagnosis, whose low sensitivity leads to false positive results. Interestingly, molecular markers have proved useful to both detect and genetically characterize invasive species.

## *Material and Methods*

Massive parallel sequencing (MPS) outputs were screened to detect (1) microsatellite markers (SSRs) for genetic population analysis and (2) single-copy genes to develop a new detection and quantification methodology, based on quantitative PCR (qPCR).

## *Results and Conclusions*

We have identified, tested, and validated a new set of polymorphic microsatellite loci using a MPS platform. After several pruning steps, 93 SSRs could potentially be amplified. Out of these SSRs, 14 were polymorphic.

Subsequently, we genetically characterized the invasive *D. polymorpha* populations of the Iberian Peninsula, including individuals

from different European and North American locations, to understand the Iberian invasion within a global context. Our results reflect a high homogeneity of the Iberian samples and suggest that *D. polymorpha* colonized the Iberian Peninsula in a recent and single invasion event.

Because *D. polymorpha* eradication is extremely difficult once the species is established, preventive detection at earlier stages is crucial to prevent its spread. Where microscopic identification of larvae failed, we used single-copy gene markers in a qPCR and found that specific Dreissenid DNA is present in filtered-water samples. Monitoring a large number of locations and using our identification methodologies as part of invasive Dreissenid management and control plans could be essential to prevention efforts in those locations where specific DNA is highly present.

# Transcriptome sequencing of cork oak and holm oak phellogenic tissue reveals biochemical, molecular and hormonal networks candidates to orchestrate cork formation and suberin biosynthesis

**Pau Boher; Olga Serra; Mercè Figueras; Marisa Molinas**

Keywords: cork cambium (phellogen), cork oak, phellem, RNA-seq, suberin biosynthesis

## *Introduction*

Because phellem (cork) is the covering tissue in plant mature (secondary) organs and healing tissues, the factors that control cork growth and differentiation are of considerable interest. Several candidate genes for cork formation have been previously identified (Soler et al. 2007, Teixeira et al. 2014). However, a comprehensive understanding of the molecular mechanisms underlying cork formation is still pending. A transcriptomic profiling was performed on the phellem of cork and holm oak trees using 454 deep sequencing and reverse-transcriptase quantitative PCR (RT-qPCR) to better understand cork biogenesis and the special features of cork oak phellem that make it unique for exploitation and commercialization.

## *Material and Methods*

First, using pyrosequencing we have generated over 11 million expressed sequence tags (ESTs) of cork RNA samples obtained from the outer bark of cork and holm oak trees. After the raw ESTs were processed, they were assembled into 16,961 contigs and functionally annotated to yield a reference phellem transcriptome. Second, we performed a comparative analysis of the EST levels of the contigs between the cork and holm oak phellem cDNA libraries. The reliability of estimates of gene expression was confirmed in 18 representative genes by microfluidic RT-qPCR. Third, the variation

in the mRNA abundance of 22 genes during the cork growing season was also evaluated by RT-qPCR.

### *Results and Conclusions*

Global functional analysis based on GO enrichment, KEGG pathways, MapMan bins, and protein conserved domain searches showed that genes involved in primary and secondary metabolism and cell wall biogenesis were more induced in cork oak while regulation and stress responses were more enriched among holm oak transcripts. Further inspection of the differentially regulated genes revealed metabolic and regulatory pathway candidates to drive energy metabolism, carbon partitioning in plastids, source-sink relationships, hypoxia responses, and suberin biogenesis in the phellem. Several phytohormone-signaling genes were differentially regulated as well. The presence of overlapping genetic mechanisms in the phellogen (cork cambium) with the cambial and apical meristems was evidenced. Moreover, a group of MADS-box transcription factors, which are known to be epigenetically controlled, were postulated as candidates to integrate endogenous and environmental signals in cork formation. Finally, the functional role of the genes analyzed by RT-qPCR and their seasonal variation is discussed in the context of cork development.

# Candidate proteins that interact with StAS1KH by two-hybrid system in potato tuber periderm

**Sandra Fernández[1], Marçal Soler[2], Pau Boher[1], Marisa Molinas[1], Jacqueline Grima-Pettenati[2], Olga Serra[1], Mercè Figueras[1]**

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Keywords: yeast two-hybrid; protein-protein interaction; periderm; AS1KH.

## *Introduction*

Periderm is the suberized external tissue that substitutes the epidermis during the secondary growth stage. It protects the plant from dehydration, pathogen entrance and radiation. How periderm development and suberization is regulated is unknown. From different transcriptomic approaches, we identified 3 regulator candidates: StAS1KH, StNAC103 and ERF3 (Ethylene response factor), all localized in the nucleus. AS1KH interacts with AS1 and has been involved in leaf morphogenesis and cambium development. To understand the regulatory function of StAS1KH and of the other regulatory candidate genes, StNAC103 and ERF, it is important to study the proteins that might interact with them.

## *Material and Methods*

The yeast two-hybrid (Y2H) system activates the transcription of a reporter gene to reveal protein-protein interactions. First, we checked that the candidate regulator gene expressed in yeast would not produce toxicity and self-activation of the reporter gene. Second, we constructed a potato tuber periderm cDNA library to detect the cDNA (prey) clones that interact with the candidate protein (bait) regulator. The clones were sequenced and the interaction in yeast was confirmed by repeating the Y2H system using the full-length coding sequence of the preys.

Protein subcellular localization was assessed by Agrobacterium-mediated transient expression in *Nicotiana benthamiana* leaves.

### *Results and Conclusions*

The construction of the library yields to $4_x10^7$ cells $ml^{-1}$. StAS1KH was the candidate regulator gene selected for the Y2H assay because it produced neither cytotoxicity nor self-activation of the reporter gene, in contrast with StNAC103 and ERF, respectively. StAS1KH allowed the obtaining of 227 cDNA prey clones, 109 of which were sequenced corresponding to 28 genes encoding putative interactors. From them, the genes codifying for a Cysteine proteinase, Isoamyl acetate, ERF2, ARC and F-box protein were selected based on i) the number of times they were isolated in the screening, and ii) their in silico-predicted localization in the nucleus. The interaction of StAS1KH and the Cysteine proteinase, Isoamyl acetate, ERF2, ARC and F-box proteins was confirmed using their full-length coding sequences.

Transient in planta expression assays showed that the Cysteine proteinase, ARC and ERF accumulate in the nucleus as StAS1kH.

Further confirmation using an in planta interaction system is needed to verify the yeast results.

# Identification of genetic elements implicated in the Adherent-invasive *E.coli* phenotype by comparative genomics

**Carla Camprubí-Font, Mireia López-Siles, Meritxell Ferrer-Guixeras, Carles Abellà-Ametller, Jesús Garcia-Gil, Margarita Martinez-Medina**

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Keywords: adherent-invasive *Escherichia coli* (AIEC); genetic element; Crohn's disease; single nucleotide polymorphisms (SNPs); molecular targets

## *Introduction*

Adherent-invasive *Escherichia coli* (AIEC) have been implicated in the aetiology of Crohn's disease but the molecular basis of its pathogenicity is not well resolved. Nowadays the identification of the pathotype is performed with time-consuming techniques based in phenotypic screening of cultured bacteria; obtaining new molecular tools would therefore be of great significance. Our aim was to identify putative genetic elements involved in AIEC phenotype to gain insight into the mechanisms of its pathogenicity and to find molecular targets for its identification.

## *Material and Methods*

Three pairs of *E. coli* strains were sequenced *de novo* by combining paired-end libraries of HiSeq and PacBio. Each pair had identical pulsed field gel electrophoresis fingerprints and virulence gene profiles, but differed in the AIEC phenotype. Each pair of strains, except for one, belongs to a different phylogroup and shares the same serogroup. Two different approaches were used to find genetic differences (SNPs) between AIEC and non-AIEC strains: the first compared each AIEC strain with its non-AIEC counterpart (Velvet and Differences) and the second compared both strains to the AIEC reference strain UM146 (SPAdes and Mauve). Functional analysis for genes with SNPs was performed.

### *Results and Conclusions*

Quality analysis indicated that the reads achieved quality scores above Q30, with an inferred base call accuracy above 99%. Genome sizes of Velvet assemblies for AIEC strains ONT:HNT-D, O6:H1-B2 and O22:H7-B1 were 4.86, 5.16 and 4.79Mb respectively. When SPAdes was used, they presented +95,362bp, +47,933bp and +30,178bp respectively. The comparative genomics for the first approach *in silico* reported 114, 80 and 31 SNPs. In contrast, the second resulted in 19, 27 and 31 SNPs respectively, and from those, 6 were found with both strategies. Although most of them were described in genes commonly distributed in all the strains, the point mutations were not AIEC-specific. The selected genes are mainly involved in metabolic processes, stress tolerance or adhesion and invasion pathways, and are therefore putatively implicated in AIEC pathogenesis.

To conclude, new genes that could be involved in the pathogenicity of AIEC have been identified. *In vitro* studies are needed to demonstrate their role. Additional coding and non-coding sequences must be analysed to identify putative genetic markers for AIEC.

# Identification of potential pancreatic cancer biomarkers based on aberrant mucin glycoforms by *in situ* proximity ligation assay

**Meritxell Balmaña[1], Sílvia Barrabés[1], Catarina Gomes[2], Raquel López-Martos[3], Celso Reis[2], Rosa Peracaula[1]**

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Keywords: biomarker; glycosylation; *in situ* proximity ligation assay (PLA); mucin; pancreatic cancer

## *Introduction*

Pancreatic cancer is a major problem in public health, due to both the lack of biomarkers and the ineffectiveness of currently available treatments. Mucins are glycoproteins that carry aberrant glycosylation moieties in malignancy expressed by epithelial cells, and thus many tumour markers in clinical use are mucins.

Our objective is to identify specific cancer related glycan epitopes on MUC1 and MUC5AC that could help to discover specific biomarkers for pancreatic cancer.

## *Material and Methods*

In the present study we have performed immunohistochemistry in 22 pancreatic adenocarcinoma tissues and 3 healthy pancreas specimens, to characterise the staining of mucins MUC1 and MUC5AC, and the cancer associated carbohydrate antigens sialyl-Lewis x ($SLe^x$) and sialyl-Tn (STn). Then, we have performed the *in situ* proximity ligation assay (PLA), a novel methodology that combines the antibodies against the mucins and against the glycan epitopes to identify specific mucin glycoforms in tumours.

## *Results and Conclusions*

Our results show the colocalization of the tumour-associated antigens $SLe^x$ and STn with the mucins MUC1 and MUC5AC in most pancreatic cancer tissues. We have shown the expression of $SLe^x$ on MUC1 and MUC5AC in the tumour cells and, remarkably, in the secretions, which could potentially reach the bloodstream. Thus, the glycoform $SLe^x$ on MUC1 or MUC5AC could be a candidate for pancreatic cancer diagnosis and should be studied in secreted fluids such as pancreatic juice or serum.

# Cultural adaptation and validation of the *Breakthrough Pain Assessment Tool* in people with cancer

**Maria do Carmo Ferreira dos Santos**

Keywords: cancer; cancer pain; breakthrough pain; pain assessment; transcultural validation.

## *Introduction*

Pain, specifically breakthrough cancer pain (BTCP), is a subjective experience that affects all dimensions of quality of life and increases patient morbidity. BTCP is characterized by a transitory increase in pain intensity when the background pain is stable and controlled with painkillers. It is estimated that between 40% and 95% of cancer patients present this symptom.

The present study aims to culturally adapt and validate Catalan and Spanish versions of the *Breakthrough Pain Assessment Tool* (BAT) in people with cancer.

## *Material and Methods*

This study aims to adapt and validate Catalan and Spanish versions of the BAT in people with cancer that are treated at the Catalan Institute of Oncology in Girona in a two-phase project.

In the first phase, the process of cultural adaptation and translation of the scales was completed. The study design followed the translation/back-translation method with two independent teams of native translators and the presentation of the survey to healthy people and patients as the cultural adaptation step.

In a second phase, the final versions in Catalan and Spanish will be validated, through the analysis of their psychometric properties: reliability and validity.

The project has been submitted to and approved by the ethical committee of the Dr. Josep Trueta University Hospital of Girona. The use of clinical information is in accordance with the ethical standards of biomedical research and the code of good scientific practice.

### *Results and Conclusions*

The process of cultural adaptation of the Catalan and Spanish translations of the English version was conducted from June to November 2015. Two teams of five people translated the BAT from English to Catalan and to Spanish before doing a re-translation. We then analyzed the differences with the original version. The BAT in Catalan and Spanish was used to initiate the pilot study before starting the final validation of the tool. The first phase will end with pilot study to prepare the final version of the BAT, which will allow the second phase of the research to begin.

# Analysis of selective cytotoxicity for tumor cells of nuclear-directed human ribonuclease variants

**Gloria García-Galindo, Jessica Castro, Anna Vert, Marc Ribó, Maria Vilanova, Antoni Benito**

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Keywords: cytotoxicity; antitumor drug; cancer selectivity; nuclear-directed ribonucleases; apoptosis

## *Introduction*

We have previously described nuclear-directed human pancreatic ribonuclease variants (ND-RNases), PE5 and NLSPE5, which are selectively cytotoxic against a panel of tumor cell lines because they possess one (PE5) or two (NLSPE5) nuclear localization signals (NLS) that direct them to the nucleus, where they can exert their ribonucleolytic activity. We have demonstrated that PE5 induces cell death through apoptosis, reduces P-gp levels in MDR cell lines and down-regulates genes involved in the deregulated metabolic pathways of tumor cells. NLSPE5 is ten-fold more cytotoxic than PE5 for tumor cells. In this work, we have studied the selectivity of PE5 and NLSPE5 for tumor cells.

## *Material and Methods*

We have determined the cytotoxicity of both ND-RNases on four non-tumor cell lines (HaCaT, CCD-18Co, HEK-293 and 1BR.3.G) and on four tumor cell lines (NCI-H460, OVCAR-8, NCI/ADR-RES and HeLa) using the MTT assay. We have studied the molecular mechanisms triggered by both ND-RNases in the CCD-18Co cell line. We have measured their effects on the cell cycle distribution by FACS and on the cell death mechanisms, i.e. the activation of caspases and the expression of several apoptosis- and cell cycle-related proteins using Western blot analysis.

### *Results and Conclusions*

Both ND-RNases are cytotoxic for non-tumor cells only at high concentration. In addition, in contrast to what is observed for treated tumor cells, they do not change the cell cycle phase distribution. Cell death is produced through apoptosis by activation of procaspases-3, -8 and -9. The effect of NLSPE5 on the expression of cell-cycle related proteins in non-tumor cells is more apparent than that produced by PE5. The overexpression of $p27^{KIP1}$ and $p21^{WAF1/CIP1}$ in non-tumor cells seems to play an essential role in promoting cell survival in non-tumor cells. These proteins can inhibit or prevent apoptosis by several mechanisms. In conclusion, the molecular events displayed by PE5- and NLSPE5-treated non-tumor cells differ from those observed in treated cancer cells and serve to explain the selectivity of the ND-RNases.

# Dosimetric impact of Acuros XB dose calculation algorithm in head and neck treatments using VMAT

**C. Muñoz-Montplet, I. Romera-Martínez, D. Jurado-Bruggeman, S. Agramunt-Chaler, J. Marruecos-Querol**

Keywords: acuros XB; deterministic dose calculation; VMAT; head and neck cancer

## *Introduction*

The ICRU report 83 provides the information required to harmonize the prescribing, recording, and reporting of intensity modulated radiation therapy. The QUANTEC reviews provide summaries of the dose/volume/outcome information for many organs. The purpose of this study is to assess the dosimetric impact of the Acuros XB dose calculation algorithm (AXB), in comparisons with anisotropic analytical algorithm (AAA) calculations, in dose prescription and dose-volume reporting to the planning target volumes (PTVs) and in constraints at organs at risk (OARs) of head and neck (H&N) volumetric modulated arc therapy (VMAT) treatments. The reporting of dose-to-medium ($D_m$) versus dose-to-water ($D_w$) is also discussed.

## *Material and Methods*

Nineteen H&N patients treated with VMAT in our institution were randomly selected. Conventional or accelerated simultaneous integrated boost was used. PTV53.9-54, PTV59.4-59.9 and PTV70 were delineated to encompass tumor and lymphatic areas at risk, tumor and high risk lymphatic regions, and tumors, respectively. OARs were outlined using international guidelines.

Plans were created for 6 MV photon beams using the RapidArc technique. Dose calculations were performed with the AAA ($D_w$) and the AXB ($D_w$ and $D_m$) for the same number of monitor units and identical beam setup.

ICRU 83 and QUANTEC parameters were analyzed. Two-tailed paired

t-tests were used to analyze the differences between AXB ($D_w$) and AAA ($D_w$) and between AXB ($D_m$) and AXB ($D_w$). F-tests were used to compare the variances of the populations. A significance level of $\alpha$=0.05 was chosen.

### *Results and Conclusions*

Results for PTVs and OARs are shown in Tables 1 and 2, respectively.

When adopting AXB for H&N VMAT treatment plans, some considerations have to be taken into account. First, absorbed doses are systematically lower when dose is reported to medium. Second, for patients with PTVs that include bone, values of $D_{2\%}$ might be higher than those calculated by AAA if dose is reported to water. This effect is counteracted by dose-to-medium reporting. Finally, for patients with OARs that include or are surrounded by heterogeneities, constraints should be reconsidered when AXB ($D_w$) is used. $D_{2\%}$, contrary to $D_{max}$, can be sensitive to the algorithm in some cases. Although dose-to-medium reporting is more consistent, further study is required.

| | PTV53.9-54 | | | | PTV59.4-59.9 | | | | PTV70 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $D_{98\%}$ | $D_{95\%}$ | $D_{50\%}$ | $D_{2\%}$ | $D_{98\%}$ | $D_{95\%}$ | $D_{50\%}$ | $D_{2\%}$ | $D_{98\%}$ | $D_{95\%}$ | $D_{50\%}$ | $D_{2\%}$ | HI |
| AAA ($D_w$) | | | | | | | | | | | | | |
| Mean | 50.5 | 51.8 | 56.4 | 72.4 | 58.4 | 59.9 | 68.4 | 73.3 | 65.7 | 67.0 | 70.6 | 73.5 | 0.11 |
| σ | 1.3 | 1.5 | 3.1 | 0.7 | 1.6 | 1.5 | 1.9 | 0.9 | 2.7 | 1.4 | 0.6 | 0.9 | 0.04 |
| AXB ($D_w$) | | | | | | | | | | | | | |
| Mean | 50.5 | 51.7 | 56.6 | 72.3 | 58.4 | 59.8 | 68.3 | 73.5 | 65.9 | 67.0 | 70.4 | 73.8 | 0.11 |
| σ | 1.3 | 1.6 | 3.1 | 0.9 | 1.4 | 1.6 | 1.8 | 1.3 | 2.0 | 1.1 | 0.5 | 1.5 | 0.04 |
| AXB ($D_m$) | | | | | | | | | | | | | |
| Mean | 49.8 | 51.0 | 55.6 | 71.2 | 57.6 | 58.8 | 67.3 | 72.1 | 64.1 | 65.9 | 69.4 | 72.4 | 0.12 |
| σ | 1.1 | 1.4 | 3.1 | 0.8 | 2.2 | 1.4 | 1.9 | 0.7 | 2.9 | 1.0 | 0.6 | 0.7 | 0.04 |
| AXB ($D_w$)-AAA ($D_w$) | | | | | | | | | | | | | |
| Mean difference | 0.0 | -0.1 | 0.2 | 0.0 | -0.1 | -0.2 | -0.1 | 0.2 | 0.2 | 0.0 | -0.2 | 0.3 | 0.00 |
| Paired t-test p | 1.00 | 0.66 | 0.78 | 0.81 | 0.58 | 0.15 | 0.27 | 0.50 | 0.40 | 0.88 | 0.13 | 0.40 | 0.70 |
| F-test p | 0.94 | 0.77 | 0.95 | 0.45 | 0.72 | 0.67 | 0.97 | 0.09 | 0.21 | 0.36 | 0.60 | 0.06 | 0.99 |
| AXB ($D_m$)-AXB ($D_w$) | | | | | | | | | | | | | |
| Mean difference | -0.8 | -0.7 | -1.0 | -1.1 | -0.7 | -0.9 | -1.0 | -1.4 | -1.8 | -1.1 | -1.0 | -1.4 | 0.01 |
| Paired t-test p | 0.00 | 0.00 | 0.03 | 0.00 | 0.03 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.48 |
| F-test p | 0.44 | 0.57 | 0.99 | 0.54 | 0.08 | 0.62 | 0.99 | 0.01 | 0.11 | 0.59 | 0.78 | 0.00 | 0.56 |

All mean and σ values in Gy except for the HI (dimensionless)

Table 1: PTVs

| | | AAA ($D_{ax}$) | | AXB ($D_{ax}$) | | AXB ($D_{ml}$) | | AXB ($D_{ax}$)-AAA ($D_{ax}$) | | | AXB ($D_{ml}$)-AXB ($D_{ax}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | σ | Mean | σ | Mean | σ | Mean dif. | t-test p | F-test p | Mean dif. | t-test p | F-test p |
| Spinal Cord | $D_{max}$ | 48,06 | 2,10 | 47,94 | 1,95 | 46,98 | 1,88 | -0,12 | 0,27 | 0,77 | -0,96 | 0,00 | 0,87 |
| | $D_{2\%}$ | 44,53 | 2,19 | 44,25 | 2,19 | 43,45 | 2,16 | -0,28 | 0,00 | 1,00 | -0,80 | 0,00 | 0,95 |
| Brainstem | $D_{max}$ | 46,23 | 4,70 | 46,05 | 4,69 | 45,40 | 4,63 | -0,18 | 0,33 | 0,99 | -0,65 | 0,00 | 0,96 |
| | $D_{2\%}$ | 42,70 | 5,08 | 42,42 | 5,02 | 41,66 | 4,94 | -0,27 | 0,15 | 0,96 | -0,77 | 0,00 | 0,95 |
| Right Cochlea | $D_{max}$ | 28,40 | 13,79 | 30,37 | 14,76 | 26,86 | 12,99 | 1,96 | 0,00 | 0,77 | -3,51 | 0,00 | 0,59 |
| | $D_{2\%}$ | 26,89 | 13,32 | 28,69 | 14,30 | 25,43 | 12,56 | 1,80 | 0,00 | 0,77 | -3,26 | 0,00 | 0,59 |
| Left Cochlea | $D_{max}$ | 24,66 | 15,43 | 26,19 | 16,55 | 23,46 | 14,68 | 1,53 | 0,00 | 0,77 | -2,73 | 0,00 | 0,62 |
| | $D_{2\%}$ | 23,40 | 14,67 | 24,80 | 15,80 | 22,19 | 13,92 | 1,40 | 0,00 | 0,76 | -2,62 | 0,00 | 0,60 |
| Jaw | $D_{max}$ | 66,30 | 8,73 | 71,44 | 11,13 | 65,23 | 8,32 | 5,14 | 0,00 | 0,31 | -6,21 | 0,00 | 0,23 |
| | $D_{2\%}$ | 61,03 | 9,90 | 64,02 | 10,63 | 59,21 | 9,31 | 2,99 | 0,00 | 0,77 | -4,81 | 0,00 | 0,58 |
| Right Parotid | $D_{mean}$ | 29,73 | 9,10 | 29,36 | 9,08 | 29,04 | 8,89 | -0,38 | 0,01 | 0,99 | -0,32 | 0,00 | 0,93 |
| Left Parotid | $D_{mean}$ | 27,27 | 7,24 | 26,92 | 7,50 | 26,48 | 7,44 | -0,35 | 0,08 | 0,88 | -0,45 | 0,00 | 0,97 |
| Oral Cavity | $D_{mean}$ | 38,82 | 11,69 | 39,30 | 11,95 | 38,49 | 11,72 | 0,46 | 0,05 | 0,93 | -0,77 | 0,00 | 0,94 |
| Larynx | $D_{mean}$ | 52,57 | 17,55 | 52,14 | 17,65 | 51,65 | 17,49 | -0,43 | 0,00 | 0,98 | -0,48 | 0,00 | 0,97 |

All mean and σ values in Gy.

Table 2: OARs

# Epigenetic silencing of TGFβ1, BCL6, KILLIN and CTSZ is related to trastuzumab resistance in HER2-positive breast cancer models

**Palomeras S, Díaz-Lagares A, Sarrats A, Crujeiras AB, Giró-Perafita A, Sandoval J, Rabionet M, Esteller M*, Puig T***

Keywords: Trastuzumab; resistance; DNA methylation.

## *Introduction*

The major clinical problem for HER2 breast cancer target therapies is the acquisition of resistance. Trastuzumab (Herceptin), the humanized anti-HER2 antibody, has been used for the treatment of HER2-positive early stage and metastatic breast cancers. However, the response rate of these patients to trastuzumab is less than 35%. Notoriously, up to 62% of the patients that respond to the initial treatment develop resistance within a year. Different cellular and molecular alterations may contribute to the development of trastuzumab resistance, including epigenetic changes. Genes critical to breast cancer tumour biology are frequently inactivated by hypermethylation of the CpG dinucleotides located in their 5'-CpG island regulatory regions. Our study aims to evaluate epigenetic differences between trastuzumab-sensitive and resistant breast cancer cell lines to determine whether they might be used as potential biomarkers to help in treatment assessment and monitoring.

## *Material and Methods*

A trastuzumab-resistant HER2-positive cell line (SK**TR**) was generated by long-term in vitro culture of SKBR3 cells in the presence of trastuzumab (Herceptin, Hoffmann-LaRoche Pharma).

A DNA methylation microarray was used to identify epigenetic differences in trastuzumab-sensitive (SK**BR3**) and -resistant cells (SK**TR**). To identify the differentially methylated CpGs, we considered differences between methylation groups of ≥60%. The functions associated with genes were analysed using the Gene Ontology.

Their differences in promoter methylation status were validated by expression techniques (qPCR) and subsequently confirmed by demethylating agent (5-aza-DC) treatment followed by qPCR.

### *Results and Conclusions*

The DNA methylation microarray data showed that different 5'-CpG island sites had altered methylation profiles when comparing SK**TR** and trastuzumab-sensitive SKBr3 cells. We selected 4 candidate genes with a hypermethylation profile in resistant vs. sensitive SKBr3 cells: TGFβ1, BCL6, KILLIN and CTSZ. The hypermethylation of these four candidate genes in SK**TR** was in agreement with the down regulation of their expression observed by PCR analysis. SK**TR** treatment with a demethylation agent restored the expression levels of all four genes to the levels of trastuzumab-sensitive cells.

These results provide a basis for future studies to validate the hypermethylation status of these genes as a predictive or monitoring biomarker of trastuzumab resistance in HER2+ breast cancer patients.

# A cytotoxic apoptin variant for cancer cells when exogenously added is intrinsically disordered

**Santiago Ruiz-Martínez[1], Jessica Castro[1], Maria Vilanova[1], Marta Bruix[2], Marc Ribó[1], Douglas V. Laurents[2] and Antoni Benito[1]**

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Keywords: apoptin; cytotoxicity; antitumor protein; protein aggregation

## *Introduction*

Apoptin (or VP3), a 121 amino acid protein hailing from the chicken anemia virus (CAV) has attracted much interest due to its ability to induce apoptosis of multiple mammalian and malignant cell lines without affecting primary and non-transformed cells. The use of apoptin is restricted by its strong tendency to aggregate. Efficient transduction to deliver the apoptin gene into tumor cells is envisaged as an alternative.

## *Material and Methods*

Here we describe the production of a soluble non-aggregating apoptin variant that has been characterized by different biochemical and biophysical approaches such as size exclusion chromatography (SEC), dynamic light scattering (DLS), circular dichroism (CD), and nuclear magnetic resonance. Additionally, we have studied the cytotoxic effect of this variant either by transfecting the gene or by exogenously adding the protein to cancer cells. We also investigated whether the apoptin variant induced cell death through apoptosis in cancer cells. This was done by FACS analysis of these cells stained with Annexin V-Alexa Fluor 488 and propidium iodide, evaluating the activation of procaspases -3, -8 and -9 and observing typical apoptosis traits through confocal microscopy.

### *Results and Conclusion*

The apoptin variant maintains the wild type protein's antitumor activity when transfected into cells and, additionally, acquires the ability to be cytotoxic when externally added. Remarkably, as observed by SEC and DLS, this variant misses the tendency of wild type apoptin to form large aggregates, facilitating its entry into the cells to induce apoptosis, which greatly facilitates the study of its biological properties. The monomeric behavior of this variant has allowed us to characterize it structurally. The CD spectra are dominated by random coil contributions. The bidimensional $^{1}H$-$^{15}N$-heteronuclear single quantum coherence (HSQC) spectrum has also been completely assigned. The analysis of these data shows that the variant is an intrinsically disordered protein that lacks a preferred conformation. Our results suggest that this apoptin variant is an interesting antitumor candidate and shed light on the structure and function of this protein.

# Effects of the D211G mutation on the membrane dynamics of the cardiac voltage-gated sodium channel β2 subunit

**Èric Cortada, Robert Serradesanferm, Helena Riuró, Ramon Brugada, Marcel Vergés**

Keywords: *SCN2B*; Nav1.5; membrane dynamics; FRAP

## *Introduction*

Cardiomyocytes are the heart muscle cells. They hold the cardiac voltage-gated sodium channel (Nav1.5) within specific domains of their cell surface, the sarcolemma. This channel, which triggers cell contraction, consists of a pore-forming α subunit and regulatory β subunits. Alterations in channel expression or function lead to channelopathies. One of these is Brugada Syndrome (BrS), a major cause of sudden unexplained death.

Recently, our laboratory discovered the first mutation causing BrS in the gene encoding the β2 subunit (*SCN2B)*. This mutation, D211G, results in a loss of channel function, an alteration leading to potentially fatal arrhythmias.

The mutation has solely been studied in the context of the α subunit, but it may also affect β2 itself. Revealing how β2 behaves at the cell surface is crucial to understanding how the mutation turns into disease.

## *Material and Methods*

We analysed the effect of D211G mutation in polarized Madin-Darby canine kidney (MDCK) cells expressing β2. For this purpose, we used biochemical approaches to study membrane targeting and endocytosis of wild-type and mutant β2. We also carried out experiments of fluorescence recovery after photobleaching (FRAP) to study β2 dynamics at the membrane. To this end, we fused β2 to yellow fluorescent protein.

### *Results and Conclusions*

The mutation has no effect on β2 surface targeting or endocytosis: both β2 wild-type and mutant localize at the apical cell surface in MDCK cells and are not endocytosed. However, we found a higher proportion of mobile β2 D211G molecules than β2 wild-type molecules (mobile fraction) by FRAP. Moreover, β2 D211G had a higher mobile fraction in partially polarized cells than in fully polarized cells. On the whole, β2 appears as a rather static integral membrane protein. In brief, we expect to yield more data concerning the specific intracellular trafficking of β2 from the Golgi to the cell surface, its half-life and rate of recycling and degradation.

Ultimately, we propose a model to explain how β2 may behave and interact with other plasma membrane molecules at the cell surface, and how the D211G mutation may affect its anchorage and dynamics at the plasma membrane.

# Control and evaluation of pain after cardiac surgery by clinical protocol to cardiac surgery

**Aaron Castanera Duro, Concepció Fuentes Pumarola, Patricia Ortiz Balludeja**

Keywords: cardiac surgery; clinical protocol; pain assessment; nursing

## *Introduction*

Pain is an alarm signal. Pain assessment and management is an important part of nursing care, during which pain is evaluated mainly by verbal communication through the Numeric Rating Scale (NRS).

Overall, between 50% and 90% of patients admitted to hospital report experiencing pain during their stay. Other authors have reported that two-thirds of patients present with moderate or severe pain during the postoperative period. Another recent study observed that 76% of postoperative patients experienced moderate to severe pain in the first 24 hours after surgery. The objective of pain control and treatment in the postoperative period is to avoid physiological and organ damage such as the associated comorbidities that have a negative effect on quality of life.

The aim of the study is to establish the extent and level of pain perceived by patients in the intensive care unit in the first 48 hours after cardiac surgery with and without cardiopulmonary bypass (CPB). Subsequently, a clinical protocol for intensive care unit (ICU) nurses (analgesia and nursing management) to adequate pain management in postoperative cardiac surgery patients is implemented and its effectiveness is evaluated.

## *Material and Methods*

We conducted a quasi-experimental, descriptive and comparative study. Our first objective was to determine the level and degree of pain in patients older than 18 who were extubated in the first 6 hours after cardiac surgery with and without cardiopulmonary bypass. The patients undergoing cardiac surgery were observed during the first 48

hours in ICU. Sociodemographic and clinical variables were collected, and the pain was assessed using the Numeric Rating Scale, to perform univariate and multivariate descriptive study.

### *Results and Conclusions*

Our preliminary results demonstrate that patients who undergo surgery without CPB have higher NRS values for pain assessment in the first few hours after surgery, compared to patients who receive CPB ($p<0.001$). Nonetheless, after 48 hours these reported values are significantly lower than those of patients with CPB ($p<0.001$).

With these data we have adapted an appropriate clinical protocol to the characteristics of patients to reduce postoperative pain. We are currently analyzing the data to evaluate the effectiveness of the protocol.

# IPF measurement in sysmex XN series PLT-F channel

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Keywords: immature platelet fraction, thrombocytopenia, PLT-F channel, reticulated platelets.

## *Introduction*

Reticulated platelets (RP) are immature platelets released from the bone marrow in a wide group of diseases; they are used as good indicators of megakaryopoietic activity. Using flow cytometry, these newly produced platelets can be distinguished from mature platelets by their RNA content. Immature platelet fraction (IPF) can be an appropriate marker in thrombocytopenias to differentiate an increased thrombopoiesis (due to increased peripheral platelet destruction) from a decreased thrombopoiesis. The Sysmex-XN analyser introduced the PLT-F channel, providing greater specificity in IPF measurement than other analysers. Furthermore, IPF values and RP values are moderately related even though they are not quantitatively identical.

The aim of this study is to know if the IPF can be a favourable parameter in differential diagnosis of thrombocytopenias.

## *Patients and Methods*

We examined 100 patients: 25 in the control group, 34 chemotherapy-treated patients (CT), 24 with chronic liver diseases (CLD), and 17 with primary immune thrombocytopenia (ITP). We used peripheral blood collected into EDTA-K3 tubes; complete blood counts were analysed using the Sysmex-XN analyser; PLT counts were done by impedance (PLT-I) and fluorescence (PLT-F) channels. IPF tests were measured by the PLT-F channel and expressed in percentages. ROC curve analysis was performed to determine diagnostic accuracy for IPF%.

*Results and Conclusions*

The platelet counts for the control, the CT, the CLD and the ITP groups were 254.58±53.8, 60.00±30.6, 78.00±15.9 and 54.29±26.6x103/uL, respectively. IPF% levels increased significantly in comparison with the control group (control group: 5.59±3.1; ITP: 19.57±7.9; P<0.001). A significant inverse correlation between IPF% and platelet count was observed in the PLT-I ($r^2$ = -0.281; p<0.01) and PLT-F ($r^2$ = -0.267; p<0.01) channels.

IPF% was clearly different in ITP compared to other thrombocytopenias (ITP: 19.57±7.9; CT: 6.47±4.1; CLD: 7.03±3.1; p<0.001). A significant AUC was obtained in ROC analysis: 0.949 (IC95%: 0.877-1.000; p<0.001). The best IPF% cut-off was 11, 7% (sensitivity 88.2% and specificity 91.5%); this parameter can be used to distinguish ITP from other thrombocytopenias.

IPF is a clinically useful parameter related to RP that can be measured using haematology analysers. IPF% has been suggested as a reliable marker for the differential diagnosis of disorders with abnormal platelet counts and the prediction of platelet recovery.

# Ultrasound-guided peripheral catheterization for difficult venous access patients

**Laia Salleras-Duran, Concepció Fuentes-Pumarola**

Keywords: peripheral venous catheterization; ultrasonography; pain

## *Introduction*

Peripheral venous catheterization is difficult in some patients, which can delay diagnosis and treatment. The use of ultrasound to locate deep veins in the upper extremities is a viable option to catheterize via peripheral veins in patients with difficult venous access.

The study objective was to assess ultrasound-guided peripheral venous catheterization in an emergency care setting, analyzing the difficulty of venous catheterization, puncture success, the number of required attempts, pain and patient characteristics.

## *Material and Methods*

A descriptive, observational study was carried out in the Emergency Department of the Hospital of Figueres.

The study included all adult patients who required peripheral venous catheterization and the nurses using the ultrasound-guided technique, whether or not the procedure was successful.

The success or failure of the technique, the number of puncture attempts and patient characteristics were recorded. Before catheterization, the nurse assessed the puncture difficulty on a 10-point Likert scale (0 absence of difficulty, 10 extremely difficult). The nurses recorded the reason why their used the ultrasound-guided technique. And patients evaluated their pain using the verbal rating scale (VRS). The Mann-Whitney U test was used for bivariate analysis.

## *Results and Conclusions*

The sample consisted of 187 patients. In 93.6% of them, the technique was successful. The mean number of attempts needed to

place a catheter was 1.25 (SD: 0.54).

The mean level of difficulty predicted was 8 (SD: 1.69). The most frequent reason for using the ultrasound was not visualizing or not being able to palpate veins (57.6%). Obesity was observed in 42% of the participants. The mean VRS score was 4.94 (SD: 2.37), 7.7 (SD: 1.7) when the technique failed and 4.8 (SD: 2.32) when it succeeded ($p$=0.002). The number puncture attempts before using ultrasound was 2.91 (SD: 1.7).

In general, the ultrasound-guided technique had a high success rate and a low rate of repeated puncture attempts. Nurses identified a high level of venous puncture difficulty before initiating catheterization. Although patients indicated the technique was painful, when it was successful, they reported less perceived pain. Obesity was a factor in predicting puncture difficulty.

The study could have incorporated random sampling, which would have been a better study design.

# Relationship between flow in the middle cerebral artery and pressure in the internal carotid artery during carotid endarterectomy

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Keywords: carotid endarterectomy, transcranial doppler, stump pressure

## *Objectives*

Analysis of correlations between flow values of the middle cerebral artery (MCA) and blood pressure in the common carotid artery (CCA) and the internal carotid artery (ICA) during carotid endarterectomy (CEA) to determine patients with high risk of intraoperative complications or new neurological events.

## *Material and Methods*

Prospective observational study of patients undergoing carotid endarterectomy for symptomatic carotid stenosis in the Josep Trueta University Hospital of Girona.

Patients were examined preoperatively and 3 months postoperatively using Doppler ultrasounds of supraaortic trunks, transcranial doppler (TCD) and MR angiographies. In addition, during the surgery we continuously monitored the velocity in the MCA with TCD and measured intraoperative pressure pre- and post-clamping in the radial artery, the ICA and the CCA.

Shunt placement is indicated if the CCA pressure after clamping is less than 40 mm Hg or the MCA velocity after clamping falls below 30% of the baseline.

The emergence of new neurological events is recorded immediately after surgery and again three months later.

### *Results and Conclusions*

Preliminary results of 22 patients. Shunt is placed in 59.5% of the patients. The Intraoperative record shows that the decrease in carotid pressure with clamping is more pronounced in CCA than in ICA (59.5 vs 36.1% P <0.05). Velocity in the MCA at the baseline was not related to baseline carotid pressures but with the pressure in the ICA after clamping (P <0.05). The intraoperative pattern of the three patients who had a new stroke is analyzed.

The intraoperative pressures measured at the ICA and CCA are not comparable. The ICA pressure seems to be the most correlated with the velocity at the MCA.

# Effects of synthetic endothelial peptides on blood-brain barrier disruption induced by ischemia and r-tPA administration

**Pau Comajoan, Carme Gubern, Gemma Huguet, Juan M Sanchez, Mar Castellanos, Elisabet Kádár**

Keywords: ischemia; recombinant tissue plasminogen activator (r-tPA); blood-brain barrier (BBB); intracerebral haemorrhage; oxygen and glucose deprivation (OGD)

## *Introduction*

According to the World Health Organization, stroke is the second cause of death in the world (2012). Despite all the recent studies, thrombolysis with recombinant tissue plasminogen activator (r-tPA) is the only approved treatment for acute ischemic stroke. However, approximately 8% of the r-tPA treated patients may suffer a symptomatic intracerebral haemorrhage due to the disruption of the blood brain barrier (BBB). Disruption of the BBB results in increased permeability and subsequent passing of erythrocytes through this structure when the functionality of the BBB components (endothelial cells, astrocytes, pericytes and basal membrane) is seriously damaged. We aim to evaluate the effects of potential protective peptides on the BBB after experimental ischemia and r-tPA administration using an *in vitro* BBB model, and an *in vivo* mouse model of haemorrhagic transformation associated with delayed administration of r-tPA in animals subjected to middle cerebral artery occlusion (MCAO).

## *Material and Methods*

In the *in vitro* model of bEnd.3 endothelial cell line and murine astrocytes co-culture, changes in cytotoxicity (LDH), permeability (FITC-BSA and TEER) and viability (MTT) have been evaluated at 0, 24 and 72h of reperfusion (with or without r-tPA and potential protective peptides) after 3h of oxygen and glucose deprivation (OGD). Furthermore, several proteins involved in BBB permeability have

been studied using Western Blot and ELISA. In the *in vivo* model, blood serum and brain tissue have been analysed using ELISA and immunofluorescence, respectively.

### *Results and Conclusions*

OGD significantly decreased the viability of bEnd.3 cells and significantly increased their permeability. r-tPA administration only modified the permeability, which significantly increased, without modifying viability. Other preliminary results suggest that OGD may modulate the expression of several proteins involved in the BBB permeability, such as caveolin-1 and ICAM-1. Additionally, tight junction proteins like occludin and ZO-1 seem to be also affected in MCAO brain tissue. In conclusion, we have set up an *in vitro* co-culture of endothelial cells and astrocytes to evaluate the effects of ischemia and r-tPA administration. Initial results suggest that r-tPA could worsen the OGD effects. Our current studies are focused on the analysis of possible potential BBB protective peptides after r-tPA administration.

# Therapeutic activity of pituitary adenylate cyclase-activating polypeptide (PACAP) in Huntington's disease

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Keywords: HD; PACAP; PAC1; synaptic plasticity

## *Introduction*

Huntington's disease (HD) is a hereditary neurodegenerative disorder characterized by early cognitive impairment. Cognitive deficits are due to hippocampal dysfunction resulting from the alteration of synaptic plasticity. The pituitary adenylate cyclase-activating polypeptide (PACAP) is a neuropeptide that promotes synaptic plasticity and memory, among other abilities, via three receptors: PAC1, VPAC1, and VPAC2. However, the therapeutic role of PACAP in HD has not been studied. Therefore, the aim of this study is to analyze PACAP as a possible therapeutic agent to restore cognitive deficits in HD.

## *Material and Methods*

The protein levels of PACAP receptors were studied in the hippocampus of R6/1 mouse models (mice expressing the exon 1 of

the human mutated huntingtin (mhtt)), in hippocampal *post-mortem* human samples, and in neural cell lines ($STHdh^{Q7}$) transfected with mhtt (htt-94Q-GFP). To study the effect of PACAP on neurite growth, we treated primary hippocampal cultures with PACAP ($10^{-7}$M). To analyze the role of the PAC1 receptor, we transfected $STHdh^{Q7}$ and mhtt ($STHdh^{Q111}$) cells with siRNA against PAC1. Finally, we performed intranasal administration of PACAP (1 µg/µl) or vehicle in WT and R6/1 mice treated at 14 weeks of age (onset of cognitive dysfunction) for 7 days and then subjected the animals to cognitive tasks.

### *Results and Conclusions*

We observed a decrease in PACAP receptors from the onset of cognitive dysfunction in R6/1 mice. The transfection of htt-94-Q-GFP also resulted in a loss of PACAP receptors indicating that the decrease in the PACAP receptors seems to be related to the expression of mhtt. The analysis of post-mortem hippocampal human samples revealed a specific reduction of PAC1, without changes in VPAC1 and VPAC2. The addition of PACAP in hippocampal cultures showed an important increase in the number of neurites and their length. Moreover, the treatment of neuronal cultures restored the expression of genes related to synaptic plasticity, such as c-fos or egr1. In addition, the transfection of siPAC1 abolishes the PACAP-related effects. Intranasal administration of PACAP blocks cognitive deficits in R6/1 mice. Our findings suggest that PACAP could be a suitable candidate to reestablish the synaptic plasticity in HD, via the PAC1 receptor.

# Reduced MEF2 transcription factor protein levels in the hippocampus of an R6/1 mouse model of Huntington's disease: role in cognitive deficits

**L. Vidal-Sancho[*1,2,3,4], S. Fernández[*2,3,4] A. Badia[2,3,4], J. Alberch[2,3,4], X. Xifró[1,2,3,4]**

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## *Introduction*

Huntington's disease (HD) is a neurodegenerative disorder caused by an abnormal expansion of a CAG codon in exon 1 of the *huntingtin* gene. HD patients and mouse models show learning and memory impairment even before the onset of motor symptoms. Deficits in hippocampal synaptic plasticity have been involved in the HD memory impairment. Several studies show that the family of transcription factors – myocyte enhancer factor 2 (MEF2) – has been involved in the memory formation process. Therefore, we studied the role of MEF2 in the hippocampus of the R6/1 mouse HD model.

## *Material and Methods*

The protein levels of MEF2 were analyzed by Western blot in the hippocampus of R6/1, an exon-1 mutant huntingtin (mhtt) mouse model. We also used a neural cell line ($STHdh^{Q7/Q7}$) to transfect mhtt (htt-94Q-GFP) and a constitutively active form of MEF2c (mtMEF2C219). To study the role of MEF2 in neurite growth, we treated primary hippocampal cultures with BIX, an inhibitor of MEF2. Finally, we intraperitoneally administered BML (an activator of MEF2) or vehicle in WT and R6/1 mice treated at 14 weeks of age (onset of cognitive dysfunction) for 3 days and then subjected them to the novel object recognition test (cognitive task).

## *Results and Conclusions*

We observed a decrease in the MEF2 protein levels from the onset of cognitive dysfunction in R6/1 mice. MEF2 also decreased in hippocampal R6/1 primary neurons and in htt-94Q-GFP-STHdh$^{Q7/Q7}$ cells. No interaction between MEF2 and mhtt was detected by immunoprecipitation and immunohistochemistry, indicating that decreased levels of MEF2 are related to its transcriptional dysregulation. This reduction was associated with the specific decrease of the isoform MEF2c. We also observed that BDNF protein levels are enhanced in cells expressing mtMEF2C219, suggesting that MEF2c could be implicated in the reduction of hippocampal BDNF levels, an important hallmark of HD. This effect was associated with a decrease in the number and length of neurites in hippocampal primary cultures treated with MEF2 inhibitor. Finally, the disinhibition of MEF2 by pharmacological treatment ameliorates the long-term memory deficits of R6/1 mice. Altogether, our results suggest that the loss of the MEF2c factor is involved in HD cognitive and synaptic deficits.

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# Embolism diagnosis in scuba diving: new autopsy guide porpouse

**J.M. Casadesús, A. Carrera, J. San, A. Puig, A. Hospital, F.J. San Miguel, F. Reina**

Keywords: scuba; autopsy; embolism; guide.

## *Introduction*

Asphyxia by drowning has been described as the main cause of death in scuba diving and dysbaric accidents. Arterial gas embolism (AGE) is also involved in the pathogenesis of this kind of deaths. Reference protocols are not currently available in our field, hindering the performance of the professionals involved.

Objective: suggest a specific autopsy technique in order to diagnose post-mortem AGE in the context of intrathoracic hyperpressure syndrome.

## *Material and methods*

The study was conducted in collaboration with the NEOMA research team of the University of Girona (UdG) and the Institute of Legal Medicine and Forensic Sciences of Catalonia (IMLCFC). We used human adult specimens donated voluntarily to the Human Anatomy Laboratory (UdG), and preserved them by freezing at -25 °C and judicial corpses of autopsy from the Forensic Pathology Service of Girona, selected by type of death, and requested and approved by the Teaching and Research Committee of the IMLCFC. The main technical parameters were carotid dissection and thoracic vertebral artery systems for isolation, without opening vascular compartments.

## *Results and conclusions*

We dissected 9 anatomical pieces, 4 corresponding to the skull and 5 to the chest. We performed an optimal cranial opening with complete meninges removal and arterial system access. In addition, we obtained

an identification, isolation and arteriovenous optimal arrangement at the thoracic level.

We used 7 scuba diving corpses; in 2 cases we obtained optimal technical operability allowing a necropsy diagnosis of AGE.

These results allow us to suggest a specific autopsy technique divided into four steps, aimed at confirming or excluding some evidence of dysbaric embolism disorder. We have demonstrated the presence of gas emboli on different organs and tissues (heart and brain) in the context of intrathoracic hyperpressure syndrome during practice scuba diving.

# Anatomical study of the lateral ulnar collateral ligament of the elbow: morphometric parameters and description of insertional morphotypes

**Diana Noriego Muñoz[1], Anna Carrera Burgaya[2], Xavier Madirolas Alonso[3], Miguel Ángel Froufe Siota[4], Francisco Reina de la Torre[2]**

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Keywords: elbow, anatomy, lateral ulnar collateral ligament

## *Introduction*

Lateral collateral ligament complex plays a key role in the stability of the elbow. However, there is no single accepted description of its morphology or of the functional anatomy of its fascicles. In this regard, the lateral ulnar collateral ligament (LUCL) lesion could be a key element of posterolateral instability.

The objective is to describe the lateral collateral ligament complex and specifically the LCUL anatomy.

## *Material and Methods*

We conducted a microdissection study in 21 adult cryopreserved elbow specimens without any previous history of osteoligamentous pathology.

A morphometric study of LCUL detailing the origin, insertion, length, thickness and insertional anatomic variations was carried out in each of the specimens.

## *Results*

In all cases, the origin of the radial ligament of the elbow and the LCUL was the humeral epicondyle. (The differentiation of its fibers at that level was impossible.)

Distally, the LCUL definition was more accurate: the fibers blend with the annular ligament to form a broad single expansion inserted along the supinator crest with a thin fibrous membrane or as two different bundles.

This insertion was combined with a fibrous membrane in 6 cases; in 9 it was a wide insertion and in 6 it was single. In terms of morphometry, the average length of the LCUL was 40.30mm, the average thickness was 1.2mm and the mean amplitude of the insertion was 6.30mm.

## *Conclusion*

The detailed anatomy of the LCUL remains controversial, mainly due to its relationship with ligamentous and capsular structures.

In relation to the literature on revised morphometric data, our study provides a more accurate description.

However, more anatomical and biomechanical analysis is needed to define more precisely the function of the lateral collateral ligament complex and the role of its elements in the pathogenesis of posterolateral elbow instability.

# Effect of sound isolation and music therapy on the comfort of mechanically ventilated patients admitted to intensive care units

**Marina Mateu Capell, Dolors Juvinyà Canal**

Keywords: sound isolation; music therapy; comfort; mechanical ventilation; nursing

## *Introduction*

Patients admitted to the intensive care unit who require mechanical ventilation are surrounded by an ambient noise level ranging from 60 to 90 dB, well above the 40 dB recommended by the World Health Organization (WHO). Sound isolation improves the patient's quality of sleep, while music therapy allows the patient to feel relaxed and comfortable. Both elements are thought to improve the patient's rest and in this way influence their recoveries.

## *Material and Methods*

We performed a randomized crossover clinical trial in the Intensive Care Unit of the Althaia Foundation hospital with intubated patients who presented hypnotic levels of Bispectral Index (BIS) ≥50 from score 0-100. In Group A this was carried out with one hour of sound isolation followed by one hour of music therapy, while in Group B the same procedure was carried out starting with music therapy and followed by sound isolation. The primary outcome was the level of comfort regarding the Bispectral Index, the Ramsay scale and the Behavior Pain scale (BPS). Noise cancelling headphones and relaxing music were used for interventions. Statistical analysis of correlated data was performed using a Generalized Estimating Equations (GEE) model.

*Results and Conclusions*

Results: Of the 130 eligible patients, 82 (40 in Group A and 42 in Group B) were randomized. The mean age was 69 years (SD = 14) and 77.3% were men. In both groups, during the music therapy and sound isolation periods, an average decrease of 4.5 points in the BIS score was observed in relation to the baseline values. The mean difference in the BIS score between the two interventions was 0.8 points (CI95%: -1.2 to 2.7). No statistical differences were observed in the Ramsay and BPS scales regarding the baseline values. No residual effects were observed between sound isolation and music therapy. Conclusions: Sound isolation and music therapy slightly improve patient comfort in terms of BIS and do not alter the values of comfort present in the initial period of the study in the BPS and Ramsay scales.

# Prevalence of healthy ageing in the urban population of 14 European countries

**Cristina Bosch-Farré, Dolors Juvinyà, Josep Garre**

Key words: ageing; healthy ageing; health promotion; SHARE

## *Introduction*

The phenomenon of population ageing is a global challenge and an opportunity to emphasize health promotion. The proportion of Europeans older than 65 years is expected to increase from the current 18.2% to 28.1% by 2050. The World Health Organization's 2012-2020 Action Plan for healthy ageing prioritizes health promotion. The most frequently used of the available models is "Successful Ageing", proposed by Rowe and Kahn. It has three domains: low probability of disease and disability; maintenance of high physical and cognitive function; and active participation in everyday life (social and productive activities). The main objective of the present study was to determine the prevalence of successful ageing in individuals aged 50 years and older in 14 European countries.

## *Material and Methods*

This cross-sectional study analysed data from the 2013 Study of Health, Ageing and Retirement (SHARE) representing 20,502 residents of urban areas (self-reported as big city, city suburbs, small city/large town) in 14 European countries. Rowe and Kahn's Model of Successful Aging was applied, as operationalized by Hank.

## *Results and Conclusions*

Global prevalence of successful ageing in European cities was 20.5%, with significant variation between countries. The highest prevalence (33.6%) was reported in Switzerland; Slovenia (16.5%), Italy (14.7%) and Spain (13%) were below the mean. Older age, lower educational level, and lower economic status were associated with a lower

proportion of successful ageing; there were no significant differences based on the size of the urban area. Stratified by the model's three domains, the results were as follows: low probability of disease and disability, 52.5%; high physical and cognitive function, 42.3%; active participation in daily life activities, 56.6%.

Significant differences were observed between countries, age groups and economic status in the ability to confront the overall challenge of population ageing. These results underline the need for European cities to strengthen health-related policies and actions to promote successful ageing.

# Multidisciplinary family intervention in primary health care based on problem solving treatment to decrease complex chronic patient use of health care services

**Laura Guerrero Caballero, Montserrat Caules Pla, Alícia Ruiz Ordóñez, Maria Puig Congost, Noelia González Camas, Maria Silvia Rubbo, Yolanda Farré Pla, Mariona Vilar Pont, Montserrat Mas Marquès**

Keywords: family intervention; complex chronic patient; health care services; problem solving; caregiver.

## *Introduction*

In the current socioeconomic context, addressing chronic diseases forces us to seek new lines of research to improve the effectiveness and efficiency of the interventions.

A family intervention can improve the family's ability to solve physical and psychosocial problems derived from the chronic disease of one of its members.

## *Material and Methods*

The main objective of this study is to analyze the effect of family intervention on a complex chronic patient's use of health care services. Secondary objectives are to analyze the effect of family intervention on a complex chronic patient's health (self-care, depression and mortality) and on their caregiver's health (depression and overload).

This study is a not a randomized clinical trial in primary health care. Two subject groups were created. The intervention group consisted of complex chronic patients assigned to cases management program from the Girona 4, Celrà and Sarrià primary health care centers and their caregivers. The control group was made up of complex chronic patients assigned to cases management program from the Girona 2 primary health center and their caregivers.

Outcome variables: mortality and use of health care services (patient

medical record); self-care (Jaarsma, inhaler compliance and Morisky-Green questionnaires); depression (Beck questionnaire); caregiver overload (Zarit questionnaire) before and after the intervention.

Family intervention based on problem solving consists of three visits in the patient home. First visit: discover, define and classify the problems, choosing those that are apt for intervention. Second visit: family meeting with different family members to generate solutions to the problems detected and to choose the most suitable solution. Third visit: evaluation.

The effect of the intervention on the health care services (hospitalizations) was analyzed by T-Student or ANOVA (independent samples); Mann-Whitney U-test and Fisher's exact or Chi-square (discrete or continuous variables, respectively, according to the groups defined in the study).

### *Results and Conclusions*

Expected results: family care decreases the use of health care services and improves complex chronic patient health (self-care, depression and mortality) and their caregiver's health (depression and overload).

Family care provides tools for families to solve problems related to complex chronic patient care, improving the efficiency of the approach.