vol. 2 - issue 2 - 2018

Journal of InterDisciplinary Research Applied to Medicine

JDReAM

ISSN 2532-7518
vol. 2 - issue 2
2018
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JDReAM

JOURNAL OF INTERDISCIPLINARY RESEARCH APPLIED TO MEDICINE

VOL. 2 ISSUE 2 (2018)

UNIVERSITÀ DEL SALENTO
2018

JDREAM. JOURNAL OF INTERDISCIPLINARY RESEARCH APPLIED TO MEDICINE
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eISSN 2532-7518
Journal website: http://siba-ese.unisalento.it/index.php/jdream

JOURNAL OF INTERDISCIPLINARY RESEARCH APPLIED TO MEDICINE

vol. 2 issue 2 - 2018

Index

Life sciences

Information and communication, Engineering and Physical sciences

Clinical sciences

Social Sciences and Humanities

Journal of InterDisciplinary Research Applied to Medicine
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2019), v. 2 i. 2, 5-25
ISSN 2532-7518
DOI 10.1285/i25327518v2i2p5
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The OMICS role in the early diagnosis of OSA

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Abstract
Obstructive sleep apnea (OSA) syndrome is a condition characterized by the presence of complete or partial collapse of the upper airways during sleep, resulting in fragmentation of sleep associated with rapid episodes of intermittent hypoxia (IH) and activation of the sympathetic nervous system and oxidative stress. (Dempsey et al. 2010, 47–112; Bradley and Floras 2003, 1671–78; Bradley and Floras 2009, 82–93). OSA is associated with a broad spectrum of cardiovascular, metabolic, neurocognitive and comorbidities that appear to be particularly evident in obese patients (Floras 2014, 3–8; Luz Alonso-Álvarez et al. 2011, 0, 2–18; Marcus et al. 2012, e714–55; Sforza and Roche 2016, 99), while affecting both sexes in a different manner and varying in severity according to gender and age (Mokhlesi, Ham and Gozal 2016, 1162–69). In recent years, studies on OSA have increased considerably, but in clinical practice it is still a highly underdiagnosed disease (Costa et al. 2015, 1288–92). To date, the gold standard for the diagnosis of OSA is nocturnal polysomnography (PSG). However, since it is not well suited to a large number of patients, also the Home Sleep Test (HST) is an accepted diagnostic method.
Currently, the major aim of the research is to identify non-invasive methods to achieve a highly predictive, non-invasive screening system for this category of subjects. The most recent reports indicate that research in this field has made significant progress in identifying possible biomarkers in OSA, using -OMIC approaches, particularly in the field of proteomics and metabolomics. In this review, we analyze a list of these OMIC biomarkers found in literature.

Keywords: Obstructive sleep apnea, OMICs Sciences, Proteomics, Metabolomics, Lipidomics

Incidence of OSA

Despite its high prevalence and the high burden of morbidity, Obstructive Sleep Apnea (OSA) remains a significantly underdiagnosed disease worldwide. The Hypnolaus study estimated that the prevalence of moderate-to-severe sleep-disordered breathing (≥15 events per h) was 23.4% (95% CI 20·9–26.0) in women and 49.7% (46.6–52.8) in (Heinzer et al. 2015, 310–8) whereas according to the American Academy of Sleep Medicine (AASM 2016), only 20% of patients are diagnosed (about 6 million out of a total of 24 million) in US. The annual cost for an undiagnosed patient is estimated to be at around $5,500 (considering direct and indirect health costs), while it drops to $2,100 per year for diagnosed patients (Pietzsch et al. 2011, 695–709). On this basis, it is evident that OSA is not only a serious health problem, but also a socio-economic problem.
OSA is also becoming dangerously frequent in children, associated with adenotonsillar hypertrophy (Capdevila et al. 2008, 274–82; Lumeng and Chervin 2008, 242–52) as well as high rates of overweight and obesity in children in western countries. These trends will have disastrous long-term consequences for global health and

life expectancy if solutions are not taken as soon as possible to correct erroneous lifestyles from the earliest age (Bixler et al. 2009, 731–36; L Kheirandish-Gozal and Gozal 2012, 713–14; Li et al. 2010, 991–97; Marcus et al. 2012, e714–55).
These data also suggest that the only way to make sustainable the costs of OSA is the prevention.

Pathogenic mechanisms associated with OSA

OSA is considered by far the most important form of sleep disturbance in breathing. It is caused by increased collapsibility or insufficiency/loss of muscular dilation capacity of the upper airways, leading to repeated pharyngeal constriction (hypopnea) or closure (apnoea), and therefore resulting in oxyhemoglobin saturation decreasing and partial pressure of carbon dioxide in arterial blood increasing (Jordan, McSharry and Malhotra 2014, 736–47).
To date, the gold standard for the diagnosis of OSA is nocturnal polysomnography (PSG). This sleep examination utilizes electroencephalography, electrooculography in both eyes, sub-mental electromyography, nasal airflow, snoring sounds, electrocardiography, thoracic/abdominal movements, pulse oxygen saturation and body position to measure various parameters. The PSG indices included were the apnoea-hypopnoea index
(AHI) and oxygen desaturation index. However, since it is not well suited to a large number of patients, also the Home Sleep Test (HST) is an accepted diagnostic method.
To restore pharyngeal patency, patients experience recurrent awakenings, resulting in fragmented sleep, followed by reduced cognitive performance and, in some cases, diurnal sleepiness episodes.
High circumference values of the neck, hips and waist, hypertension (HTN), usual nocturnal snoring, as well as presence of a diabetic condition and a high body mass index (BMI) are often coexisting conditions in the OSA patient at the time of diagnosis. Among these comorbidities, obesity is often the worst aggravating factor, leading to the activation of molecular mechanisms that, if not effectively and early identified, may worsen the overall clinical pattern of OSA.
In the literature, substantial evidence suggests that OSA increases oxidative and inflammatory processes on animal models and humans. Notably, chronic hypoxia-reoxygenation cycles due to intermittent hypoxia (IH) have been shown to promote the activation of inflammatory pathways (Lavie 2003, 35–51), such as increased pro-inflammatory cytokine production, metabolic dysregulation and insulin resistance (Christou et al. 2003, 105–9; Ciftci et al. 2004, 87–91). In addition, several markers of reactive oxidative species (ROS) are increased in OSA, amplifying inflammatory cascades and endothelial dysfunctions, such as the development of atherosclerosis, as well as promoting central nervous system dysfunctions (Lavie 2003, 35–51; Wang, Zhang and Gozal 2010, 307–16; Shelley X.L. Zhang, Wang and Gozal 2012, 1767–77; Zhou et al. 2016, 9626831).
Many clinical researches have clearly demonstrated that acute IH, associated with the activation of the sympathetic nervous system and strictly linked to a persistent condition of oxidative stress, represent the pathogenetic modalities for the manifestation of cardiovascular comorbidities in OSA, such as systemic arterial hypertension, left ventricular hypertrophy, cardiac rhythm alterations and, following associated early alterations of the vasal endothelium, with increased risk of cerebral stroke and myocardial infarction. (Alchanatis et al. 2002, 1239–45; Ameli et al. 2007, 729–34; Amin et al. 2002, 1395–99; Brooks et al. 1997, 106–9; Lesske et al. 1997, 1593–1603; Varadharaj et al. 2015, 40–47; Jose M Marin et al. 2005, 1046–53; Mason et al. 2012, 1791–98)
Other studies have shown clear and solid associations between OSA and HTN (Nieto et al. 2000, 1829–36; Peppard et al. 2000, 1378–84; Phillips and O'Driscoll 2013, 43–52; Ren et al. 2016, 1264–70), type II diabetes (Lai et al. 2016, 543–51; Plíhalová, Westlake and Polák 2016, S79-84; Reichmuth et al. 2005, 1590–95), stroke (Arzt et al. 2005, 1447–51; Campos-Rodriguez et al. 2013, 99–105; Ifergane et al. 2016, 1207–12), heart failure (Pearse and Cowie 2016, 353–61; Cowie 2016, 255–65; Shahar and Whitney 2001, 19–25), coronary heart disease (Loo et al. 2014, 631–36; José M. Marin et al. 2012, 2169–76; Selim, Won and Yaggi 2010, 203–20), cardi-

ac arrhythmias (Vizzardi et al. 2017, 490–500), cancer (Campos-Rodriguez et al. 2013, 99–105), metabolic, neurodegenerative and respiratory diseases (Al Lawati, Patel and Ayas 2009, 285–93; Caples, Garcia-Touchard and Somers 2007, 291–303; Tahrani, Ali and Stevens 2013, 631–38; Young et al. 2008, 1071–78).
However, the factors determining the damage in a given OSA patient are not yet well defined, so research is still ongoing (Bhattacharjee et al. 2011, 313–23; Leila Kheirandish-Gozal and Gozal 2013, 338–43; Tan, Kheirandish-Gozal and Gozal 2014, 474–80).

Searching for new biomarkers

Given the difficulty of applying Home Sleep Test (HST) to the population as a screening system due to high cost and examination timing, researchers are currently focusing on identifying new biomarkers for the early diagnosis of OSA (Mullington et al. 2016, 727–36).
In 1998, the National Institutes of Health Biomarkers defined a biomarker as an objectively measured feature evaluated as an indicator of normal biological or pathogenic processes, or either pharmacological responses to a therapeutic intervention (Strimbu and Tavel 2010, 463–66). Biomarkers can therefore provide information for diagnosis, prognosis, regression or response to treatment. In this scenario, the -OMIC sciences, such as genomics, transcriptomics, proteomics, and metabolomics, have been widely applied for finding new biomarkers in various disease mechanisms. The identification of such molecules involved in the pathogenesis of OSA patients, can facilitate early diagnosis and help to understand the complex mechanism of this disease.
In the case of sleep disorders and lung diseases, traditional biomarker research techniques have proved to be not particularly performing. Studies based on proteomics (Zheng et al. 2015, 7046; Jurado-Gamez et al. 2012, 139–46; Gozal et al. 2009, 1253–61) and metabolomics (Auffray et al. 2010, 1410–16; Davies et al. 2014, 10761–66; Weljie et al. 2015, 2569–74; Giskeødegård et al. 2015, 14843) techniques instead, have proven to be more sensitive, although, to date, the number of molecules potentially available for clinical application in the OSA context is still limited.. The development of new technologies is therefore necessary, also to provide a greater understanding of the biochemical mechanisms involved in OSA.

Proteomics Approach

The study of the proteome in OSA patients has been largely assessed. Many studies have reported that OSA patients express increased levels of mediators of the systemic inflammatory response. Zhang et al. (Huina Zhang et al. 2018, 97–108) have performed, for the first time, a proteomic approach to detect protein profiles of serum extracellular microvescicle proteins in OSA patients and in a chronic IH rodent model. Extracellular microvescicles are vescicles released from cells into the extracellular fluid environment, including serum. Their potential utility in clinical diagnosis is well documented, since vescicles are reported to reflect the physiological or pathological status of the tissue from which they arise. They found 4 differentially expressed proteins in serum extracellular microvescicles of OSA patients compared to control: C-reactive protein (CRP), Haptoglobin (HP), Fibronectin (FN1), and Platelet factor 4 (PF4). In addition, Nadeem et al., (Nadeem et al. 2013, 1003–12) have confirmed expressed level of CRP and other systemic inflammatory mediators, including intercellular adhesion molecules (ICAM), coagulation factors (factor VIII, tissue factor), and significant increase in serum levels of tumour necrosis factor alpha (TNF-α), interleukin 1β (IL-1β) and interleukin 6 (IL-6) were observed in patients with OSA. The excessive infiltration of inflammatory cells is also highlighted by the formation of subepithelial edema in OSA patients seen by histology. Among these proteins, circulating CRP is an important predictive factor of cardiovascular risk involved in the onset and progression of atherosclerosis. Its pro-inflammatory and proaterogenic properties have been found in endothelial cells, muscle cells, both smooth and striated, and macrophages. Its levels, as well as those of IL-6, are strongly associated with oxidative stress or anoxia (Nena et al. 2012, 181–86; Huina Zhang et al. 2018, 97–108). A similarly important role in the clinical picture of the OSA patient is the high level of TNF-α ob-

served; it is in fact a pro-inflammatory cytokine with an important role in the defence of the host, which at the same time mediates the onset of a series of pathological processes such as atherosclerosis, septic shock and autoimmune diseases. The release of TNF-α is mediated by IL-6, as well as by other pro-inflammatory cytokines such as IL-2, IL-2, IFN-γ and by TNF-α itself through a positive feedback process (Aihara et al. 2013, 597–604; Dyugovskaya et al. 2011, 154–62).

A whole-genomic microarrays recently carried out by Yung-Che et al., has shown angiomotin (AMOT), pleckstrin homology, MyTH4 and FERM domain containing H3 (PLEKHH3), adenosine deaminase RNA specific (ADAR), baculoviral IAP repeat containing 3 (BIRC3), and galectin 3 (LGALS3) proteins over-expressions in the treatment-naïve OSA patients (Chen et al. 2017). LGALS3 has shown to be involved in cancer, inflammation and fibrosis, heart disease, and stroke. Studies have also proved that the expression of galectin-3 is implicated in a variety of processes associated with heart failure, including myofibroblast proliferation, fibrogenesis, tissue repair, inflammation, and ventricular remodelling (Henderson and Sethi 2009, 160–71; Sharma et al. 2004, 3121–28; Liu et al. 2009, H404–12).

Expression of AMOT in endothelial cells and its level is associated with proliferation and invasion of breast tumours (Lv, Lv and Chen 2015, 1938–46).

ADAR are double chain RNA editing enzymes responsible for post-transcriptional modification of mRNA transcripts by changing the nucleotide content of the RNA. The conversion from A to I in the RNA disrupt the normal A:U pairing which makes the RNA unstable (Samuel 2011, 180–93). ADAR is considered to be involved in the insurgence of cancer (9). Studies in sleep field, revealed also that the ADA G22A polymorphism (c.22G>A, rs73598374) is associated with fewer awakenings throughout the night, and a higher duration of slow wave sleep (SWS), as compared to the normal ADA G22G genotype (Milrad et al. 2014).

BIRC3 is a downstream effector of the ubiquitous hypoxia-inducible factor (HIF-1α) that is involved in pro-survival and inflammatory responses induced by the docosahexaenoic acid/neuroprotectin D1 pathway under oxidative stress in an ischemia-reperfusion stroke model. HIF-1 α functions as a principle regulator activity of cellular and systemic homeostatic response to hypoxia. This heterodimer composed of an alpha and a beta subunit can activate the transcription of many genes, including those involved in energy metabolism, apoptosis, angiogenesis, and other genes whose protein products increase oxygen delivery and facilitate metabolic adaptation to hypoxia. Since many studies have shown that OSA is associated with an imbalance between oxidant production and antioxidant activity, this fact, combined with an overabundance of oxidants can be linked to the multifactorial aetiology of metabolic disorders, including insulin resistance (Henriksen, Diamond-Stanic and Marchionne 2011, 993–99).

Almendros et al. (Almendros et al. 2018, 272) examined the correlation between HIF-1α factor and vascular endothelial growth factor (VEGF) expression in patients with cutaneous melanoma. Interestingly, they found that in a large prospective study, the expression of HIF-1α was an independently factor associated with nocturnal IH measures of respiratory disturbance during sleep in patients affected by cutaneous melanoma (Almendros et al. 2018, 272), this means that it has a significant contribution to the disease. Notably, the risk of melanoma was significantly higher in patients with OSA (HR = 1.14, 95% CI 1.10-1.18), along with pancreatic and kidney cancer (Gozal, Ham and Mokhlesi 2016, 1493–1500).

In the recent years, other potential association between OSA and cancer have been reported, principally ascribed to IH effect on tumour biology (Gozal, Farré and Nieto 2016, 43–55; Gozal, Ham and Mokhlesi 2016, 1493–1500; Martínez-García, Campos-Rodriguez and Barbé 2016, 451–63; Almendros et al. 2014, 593–601).

A significant correlation between OSA and increased cardiovascular risk and HTN is strongly reported in literature (Sjöström et al. 2002, 602–7; Drager et al. 2010, 1135–39; Gami et al. 2004, 364–67; Sin et al. 1999, 1101–6). Mass-spectrometry was performed on salivary samples of OSA patients with cardiovascular diseases (CVD) compared to non-CVD OSA patients (Zheng and Li 2014, 7046). A panel of 11 biomarkers were identified to be differentially expressed between the two groups. They found that the alpha-2-HS-glycoprotein (AHSG) pep-

tide level was significantly lower in OSA-CVD group compared to non-CVD group. Reduced level of AHSG was already found in severe OSA patients (Barceló et al. 2012, 1046–48) and at metabolic level (Dyugovskaya et al. 2011, 154–62) (see next chapter). AHSG protein is synthesized by hepatocytes and it is involved in severel process such as brain and bone formation and endocytosis. Interestingly, lack of this protein is involved in leanness.

Another studied association between OSA and HTN was performed by Koyama et al. (Koyama et al. 2009, 1107–11). They studied 266 OSA patients on respect to the Angiotensin converting enzyme (ACE) gene. This gene contains an insertion/deletion polymorphism on the intron 16 characterized by a 287-bp DNA sequence (Alhenc-Gelas et al. 1991, 33–39). They showed that ACE II homozygote genotype protects from severe OSA in HTN patients.

Metabolomics Approach

The field of metabolomics, and the consequent search for potential biomarkers in OSA patients, is beginning to be explored only in recent years. The lipidomic profile in OSA patients reported in literature, mainly reveals alteration in the phospholipid biosynthesis and fatty acids expression. One of the major study through mass-spectrometry technique has allowed to identify, both at a serum and urinery level, as many as 103 proteins differently expressed in adult OSA patients compared to controls, all potentially associated with imbalances in lipid metabolism and alterations in the vascular system (Jurado-Gamez et al. 2012, 139–46). Among phospholipids, glycerophosphocholines (PC), lysophosphatidylcholines (LPE), glycerophosphoethanolamines (PE), lysophosphatidylethanolamine (LPA), phosphoserine (PS), and lysophosphatidic acids, along with glycerophosphates (PA), monoacylglycerophosphocholines, lyso-phosphocolyne (LPC) and sphingomyelin (SM) classes were found to be up regulated in patients with OSA compared to control (Ferrarini et al. 2013; Lebkuchen et al. 2018, 11270). Increased PC expression at salivary level was also reported through a LC-MS/MS methods (Kawai et al. 2013; Engeli et al. 2012, 2345–51).

Fatty acids alteration was also detected in OSA. Among those that significantly increased among OSA group compared to normal subjects, circulating anandamide (AEA), 2,4-dihydroxybutyric acid, 2-hydroxy-3-methylbutyric acid, 3,4-dihydrxoybutyric acid, 6-aminocaproic acid, pentanoic acid, and glyceraldehyde, 3-methyl-3-hydroxybutyric acid, and 4-hydroxypentenoic acid were up-regulatetd, whereas the bile acid and glycochenodeoxycholate-3-sulfate (GCDCA-3-sulfate) decreased (Papandreou 2013, 569–72, Kawai et al. 2013; Engeli et al. 2012, 2345–51). Other groups, through GC-LC techniques, found that palmitoleic and oleic acid levels were lower, while stearic acid levels were higher in the tonsillitis tissue of infant control subjects, compared to the hyperplastic tissue typical of the diseased counterpart (Ezzedini et al. 2013, 1008–12).

Other research groups observed that in OSA patients, levels of 1/2-arachidonoylglycerols (AG), and oleoyl ethanolamide (OEA) in plasma where higher when compared to controls. It was interesting to note that also AA arachidonic acid (AA) concentrations and eicosanoids (Ferrarini et al. 2013; Lebkuchen et al. 2018, 11270) were up-regulated in OSA patients, suggesting a role for the endocannabinoid system in regulating blood pressure in patients with high risk OSA for HTN and CVD (Kawai et al. 2013; Engeli et al. 2012, 2345–51).

The endocannabinoid system is, in fact, based on lipid molecules produced by the body in response to various stimuli that bind specific membrane receptors associated with the protein G and called cannabinoid receptors type 1 and 2 (CB1 and CB2) (Pagotto, Vicennati and Pasquali 2008, 74S-82S). The endocannabinoid system represents a neuromodulation system, playing an action in the control of pain at the level of the central nervous system, in the regulation of cell proliferation processes and in the modulation of the immune response. Interestingly, it also seems to play a role in the mechanisms that modulate appetite and therefore obesity (Di Marzo et al. 2001, 822–25; Croxford and Yamamura; Marsicano et al. 2002, 448–56; Engeli et al. 2012, 2345–51; Salzet et al.

2000, 4917–27). The endocannabinoid system also plays an important role in the release of adipokines. Recent research has shown that the pharmacological blockade of CB1 by an antagonist, named Rimonabant, stimulates the release of adiponectin, that is normally inhibited. Adiponectin is a circulating hormone secreted by adipose tissue, with antiaterogenic and antidiabetic properties that can reduce liver glucose production, as well as suppress lipogenesis and activate the oxidation of fatty acids (Matias et al. 2006, 3171–80; Jbilo et al. 2005, 1567–69). The endocannabinoid ways of regulating metabolism are still only partially understood, despite the fact that their role in controlling hunger and satiety acts mainly in hypothalamic structures through the activation of neurons capable of producing neuropeptides with pressizing and anorexic action (Park and Bloom 2005, 228–33). Alterations to the endocannabinoid system therefore affect and alter the energy metabolism of the body and the homeostasis of lipids, as suggested by Di Marzo and Matias, the first to formulate the increasingly true hypothesis that obesity can be associated with a pathological hyperactivation of the endocannabinoid system (Di Marzo and Matias 2005, 585–89). All these conditions can be associated with an increased risk of cardiometabolic diseases such as type 2 diabetes, dyslipidaemia, arterial hypertension, myocardial infarction and stroke, conditions normally found in OSA patients.

Mediators involved in systemic inflammatory response and oxidative stress were also reported in OSA. Among the metabolites associated with oxidative stress, the 15-F2t-urinary isoprostane, one of the most sensitive metabolites correlated to lipid peroxidation, is positively linked to the thickness of the intimo-media carotid tunic (Paci et al. 2000, S87-91). These molecules were shown to be a specific, chemically stable, quantitative marker of oxidative stress in vivo. In particular, F2t-isoprostanes are prostaglandin isomers synthesised in vivo through the free radical catalysed peroxidation of AA in biological membranes, independently of the activity of cyclo-oxygenase (Morrow et al. 1990, 9383–87). Increased urinary excretion or plasma concentrations of 15-F2t-isoprostane has been observed in many conditions including smoking, diabetes, and cardiovascular diseases (Nonaka-Sarukawa et al.).
Another important biomarker of oxidative stress, Malondialdehyde (MDA), is significantly higher in concentrations detected in patients with OSA than in controls (Denis Monneret et al. 2010, 619–25; Dikmenoğlu et al. 2006, 255–61). MDA is the result of lipid peroxidation of polyunsaturated fatty acids. It is an important product in the synthesis of thromboxane A2 in which cyclooxygenase 1 or cyclooxygenase 2 metabolizes AA into prostaglandin H2. ROS degrade polyunsaturated lipids, forming MDA (Gawel et al. 2004, 453–55). This compound is a reactive aldehyde and is one of many reactive electrophilic species that cause toxic stress in cells, reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts and can be used as a biomarker to measure the level of oxidative stress in an organism (Pryor and Stanley 1975, 3615–17; Marnett 1999, 83–95).
Arguably, the tricarboxylic acid cycle (TCA) and its mediators, tend to increase in OSA (Xu et al. 2016, 30958), suggesting augmenting of the oxidative stress.

Among Metabolites that find space as pro-inflammatory markers, Stanke-Labesque et al. have found Leucotriene E4 (U-LTE4), an inflammatory molecule associated with cysteinyl leukotriene production, whose elevation in urinary concentration has been demonstrated in patients with OSA. Recently, Gautier-Veyret and his group have shown that this pathway activation contributes to OSA-induced atherogenesis and its blockade could represent a new therapeutic target for reducing CVD (Gautier-Veyret et al. 2018, 311–19). It is also interesting to note that Continuous Positive Airway Pressure (CPAP) treatment, a respiratory ventilation method mainly used in the treatment of sleep apnea, reduces the urinary concentration of U-LTE4 by up to 22%, but only if the treatment is carried out in patients with a normal BMI (Stanke-Labesque et al. 2009, 364-370.e2).
Arguably, CPAP treatment reduces also serum levels of Homocysteine (Hcy) by almost 30%, that, along with plasma levels, were found to be significantly higher in patients with OSA compared to those of the controls (Ezzedini et al. 2013, 1008–12; Papandreou 2013, 569–72, Fletcher et al. 1987, 35–44; Findley et al. 1988,

556–61). In addition, neural-like cell exposure to Hcy for a period of 5 days resulted in a 4.4-fold increase in ROS production (Currò et al. 2014, 1485–95). Hcy is known to mediate adverse effects on cardiovascular endothelium and smooth muscle cells with resultant alterations in subclinical arterial structure and function (Ganguly and Alam 2015, 6), leading to CVD and its complications, such as heart attacks and strokes (Baszczuk and Kopczyński 2014, 579–89). Moreover, hyperhomocysteinemia leads to enhancement of the adverse effects of risk factors like HTN, smoking, lipid and lipoprotein metabolism, as well as promotion of the development of inflammation (Baszczuk and Kopczyński 2014, 579–89). Another study demonstrated that Hcy is capable of initiating an inflammatory response in vascular smooth muscle cells by stimulating CRP production, which is mediated through NMDAr-ROS-ERK1/2/p38-NF-κB signal pathway (Pang et al. 2014, 73–81). CRP expression was also found to be altered in the proteome of OSA patients (see previous chapter).

Some studies also suggest that elevated Hcy levels may be associated with alterations in mental health such as cognitive impairment, dementia, depression, Alzheimer's and Parkinson's disease (Faeh, Chiolero and Paccaud 2006, 745–56; Carmel and Jacobsen 2001) through its capacity to act as a neurotransmitter. In particular, Hcy may act either as a partial agonist at glutamate receptors or as a partial antagonist of glycine co-agonist site of the NMDA receptor, therefore in the presence of normal glycine levels and normal physiological conditions, Hcy does not cause toxicity but in case of a head trauma or stroke, there is an elevation in glycine levels in which instance the neurotoxic effect of Hcy as an agonist outweighs its neuroprotective antagonist effect. This neuronal damage following a stroke has been attributed to the over stimulation of excitatory amino acids such as glutamate and aspartate through activation of NMDA receptors (Ganguly and Alam 2015, 6; Carmel and Jacobsen 2001). Ganguly et al. (Ganguly and Alam 2015, 6) have investigated how Hcy is able to selectively stimulate the release of these excitatory amino acids in stroke and they concluded that they may trigger the release of catecholamine, resulting into detrimental effect in brain and cardiovascular system. Interestingly, in OSA patients, glutamate metabolites were also found to be significantly altered (Xu et al. 2016, 30958).

The study of catecholamine metabolites and derivatives as potential predictors of the onset of the pathological process seems particularly promising. Fletcher et al. (Fletcher et al. 1987, 35–44), for example, observed that norepinephrine (NE) and normethanephrine levels were significantly higher in the urine of patients with OSA than those found in obese HTN controls, as well as epinephrine (E) levels, at the plasma level (Findley et al. 1988, 556–61). Data subsequently confirmed by Paci et al. (Paci et al. 2000, S87-91), who also found higher levels of dopamine (DA) in the comparison of 10 male patients with OSA and 11 controls. HPLC observations revealed a significant increase in all urinary catecholamine in OSA children, and that the levels of NE and E during the night are strongly related to the severity with which they manifest the altered phenotype. Paik et al (Paik et al. 2014, 517–23), after studies carried out using GC-MS to detect metabolites of urinary neurotransmitters, demonstrated that homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), both dopamine metabolites, were increased in sleepy patients with OSA, suggesting that excessive daytime sleepiness in these subjects is probably caused by an increase in night-time activity of the dopaminergic and sympathetic systems (O'Driscoll et al. 2011, 483–88). Although this theory seems intriguing, the results of several other studies question it. Paci et al. have reported that E and DA levels did not vary significantly between OSA patients and controls. In addition, the results of the studies of Gislason et al, found 5-hydroxyindo-lacetic acid (5-HIAA), HVA and 3-methoxy-4-hydroxypheglicolenyl glycol (MHPG) in the cerebrospinal fluid of 15 patients with OSA and 18 controls; however, even in this case, the levels of all these biomarkers were similar in patients with OSA and control subjects (Paci et al. 2000, S87-91; Gislason et al. 1992, 784–86).

The inconsistency of the results obtained from the studies on catecholamine metabolites in patients with OSA may be due to various factors

such as the heterogeneity of the analytical platforms used by the various research groups, the different biological matrices taken into account, the small size of the samples, and the different protocols used for sample collection. Elements that may also affect the reproducibility of studies.

The first studies aimed at finding differentially expressed metabolites at the urinary level in children with OSA, was carried out by Krishna et al. (Krishna et al. 2006, 221–27). They adopted a mass-spectrometry technique on a cohort of 22 subjects, who demonstrated an alteration in the glomerular and tubular filtration of the kidneys, compared to the healthy counterpart. High levels of proteins such as jasmine, perlecan (a heparan sulfate proteoglycan), albumin, and immunoglobulin were detected in urine. Result which suggested increased catabolic activity of some proteins in OSA patients (Krishna et al. 2006, 221–27). Also in the same period, Shah et al. identified at the silky level, three proteins of 5896, 3306 and 6068 kDa respectively differently expressed in pathological children, capable of discriminating the latter from healthy patients with 90% specificity and 93% sensitivity (Shah et al. 2006, 466–70). Three years later, Gozal et al., through a method based on the use of 2D-DIGE-MS, were able to identify 16 metabolites differently expressed in the urine of OSA patients compared to controls. In particular, the analysis of concentrations of some of these, including uromodulin, urocortin-3, orosomucoid-1, and kallikrein, were able to identify the pathogenic phenotype with a sensitivity of 95% and even a specificity of 100% (Gozal et al. 2009, 1253–61).
The contribution of Seetho et al. and Zeng et al. in the field of research into potential OSA biomarkers was extremely interesting, with the first, focusing on the research of polypeptides using the urine of obese OSA patients used as a biological matrix, and the second, looking for proteins differently expressed between OSA patients suffering from CVD and not, in saliva. The work of the two groups allowed identifying 27 potential biomarkers, fibrinogen alpha chain (FGA), tubulin alpha-4A chain (TUBA4A) and AHSG. More specifically, AHSG has been shown to be expressed at lower levels in OSA frameworks associated with changes in cardiovascular function (Seetho et al. 2014, 1104–15; Zheng and Li 2014, 7046).

Alteration in the amino acid biosynthesis were also reported in OSA through metabolomics approach. Xu et al. identified 21 differentially expressed urinary metabolites among simple snoring group and control, including aspartylserine, isoleucine-threonine (Ile-Thr), and methionine, whereas levels of 3-hydroxyanthranilic acid and 5-hydroxytryptophan decreased. Hydroxyprolyl-methionine, hypoxanthine, Ile-Thr, indole-3-acetamide, isoleucine, lactic acid, myo-inositol, pentanoic acid, threitol, threoninyl-methionine, trimethylamine N-oxide (TMAO), uridine, and valine were consistently higher or lower (Xu et al. 2016, 30958). Other groups have also reported that methylcysteine and serine decreased in OSA condition (Kawai et al. 2013; Engeli et al. 2012, 2345–51).

The metabolomics profiling of spermine biosynthesis, indoles and tryptophan metabolism, tyrosine metabolism as well as porphyrin metabolism were also altered significantly (Xu et al. 2016, 30958; Papandreou 2013, 569–72).

Conclusions

OSA is characterized by recurrent episodes of collapse of the upper airways during sleep, which are reflected in a desaturation of haemoglobin that leads to the awakening of affected subjects. The chronic IH registered in this condition, leads the body to enact molecular adaptations to the low-oxygen conditions to which it is subject (Young et al. 1993, 1230–35). Despite this, sleep fragmentation results in a dangerous condition of excessive sleepiness during the rest of the day. In addition to the long-term problems that are listed, this sleep fragmentation entails a daily danger for the individual linked to the increased risk of road or work accidents. The body responds to chronic fatigue through compensatory mechanisms that evoke inflammatory responses, hyperactivation of the sympathetic system and alteration of endothelial function, like regulation of tight junctions; these events have an important role in promoting the onset of atherosclerosis and, in the long run, of cardiovascular and cerebrovascular diseases (Nadeem et al. 2013, 1003–12).

Recent studies show also a significant correlation between OSA and metabolic and neurocognitive risk (Sjöström et al. 2002, 602–7; Drager et al. 2010, 1135–39; Gami et al. 2004, 364–67; Sin et al. 1999, 1101–6), as well as an association with cancer mortality.
In literature, proteomics and metabolomics approaches were used to detect change in physiological or pathological status of OSA patients compared to controls, in order to find out new mediators that can be used as biomarkers of the disease. Notwithstanding OSA is a 'quite new' emerging disease, there are lots of proteins and metabolites that arise during disease, in particular those involved in inflammation and oxidative stress, in line with the clinical IH that patient undertaken in OSA disease.
Lipid dismetabolism in OSA reflects alteration in phospholipids biosynthesis, steroidogenesis and fatty acids expression. This may influence the cell membrane formation, incrementing lipid uptake, atherogenesis and inflammation. In addition, alterations in amino acids, nucleic acid and some mediators that act as neurotransmitters, such as Hcy and the endocannabinoid system were seen in OSA patients, suggesting an increased risk of cardiometabolic diseases such as type 2 diabetes, dyslipidaemia, arterial hypertension, myocardial infarction and stroke, conditions normally found in OSA patients.

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Journal of InterDisciplinary Research Applied to Medicine
JDREAM
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2019), v. 2 i. 2, 27-33
ISSN 2532-7518
DOI 10.1285/i25327518v2i2p27
http://siba-ese.unisalento.it, © 2018 Università del Salento

Can graph theory discriminate between aptamer-protein configurations?

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Abstract
Graph theory has been extensively applied in the study of proteins, although few applications still exist on biomolecules like aptamer-protein complexes, whose structure is in silico obtained. Aptamers represent a challenging field of research, especially for their involvement in therapy, diagnosis and early detection of illness; furthermore, the in vivo procedures to synthetize them are quite expensive and more time-consuming than those in silico. This paper is focused on the question if and how general network parameters are able to anticipate some features of those biomolecules. The rationale resides in the fact that, studying a large set of aptamer-angiopoietin complexes, two different types of conformers are manifest. Both types could be present in a real sample with their relative amount reflecting, in a typical population shift scenario, the affinity of the whole sample.

Keywords: graph theory, in silico structure of biomolecules, aptamer-angiopoietin complex.

Introduction

In recent years, several techniques have been developed for the early diagnosis and the ongoing follow up of several fatal diseases. The main prerequisites are: to be minimally invasive, to make use of biodevices based on nanosized materials, to show high biocompatibility and long-term stability. Aptamers are small fragments of ssDNA or RNA, entitled to play a primary role in this challenge. They are artificially assembled for achieving high binding affinity to their targets (from proteins to simple ions). Furthermore, low dimension, short half-time, low immunogenicity and production costs make them formidable competitors of antibodies in both diagnosis and therapy.
Nevertheless, it has to be pointed out that acceptance and use of aptamers in industry and by (bio)pharmaceutical companies is still rare (Famulok and Mayer 2014). The reason is primarily linked to the lack of information concerning the binding mechanism with target, intrinsically interesting, since a single referencing model does not exist and different possible scenarios have been drawn to explain the formation of aptamer-receptor complex, including conformational shift, induced fit, lock and key (Koshland 1995; Kinghorn et al. 2017). Furthermore, the in vivo exploration (Tuerk and Gold 1990) is quite difficult and can be extremely time-consuming. Public data, such as the European Bioinformatics Institute (EMBL-EBI https: //www.ebi.ac.uk), provide free and open access to a range of bioinformatics applications for sequence analysis, but, mainly for aptamers, crystallographic characterization is still in its infancy.
In parallel with the in vitro techniques, many computational procedures have been developed for predicting 3D structure from sequence information. In general, aptamer-protein complexes are obtained passing through two main steps: first, aptamers have to be folded, thus the folded (3D) sequences have to be docked with the protein. Docking tools provide a ranking for the obtained configurations, taking into account many parameters, such as the interaction energies between the two molecules, the desolvation and solvation energies associated with the interacting molecules and the entropic factors that occur upon binding (Trott and Olson 2010).
However, the ranking assignment continues to be an open question (Kaufmann et al. 2017; Cataldo et al. 2018). In a recent investigation

(Cataldo et al. 2019), a new estimate was proposed to complement the computational ranking outcomes, based on maximum likelihood criteria of the topological and electrical properties of aptamer-protein complexes. It was applied on a set of anti-angiopoietin(Ang2) aptamers, whose performances are known from the experiments (Hu et al. 2015). From the analysis, two principal types of conformers were identified and a deep discussion of the possible scenarios linked to those results was performed. The present paper aims to formulate a novel and simplified procedure to analyse all those different conformers, looking at topological characteristics of the networks representing the biomolecules. We show that important features can be extracted, another type of arrangement aptamer-Ang2 is observed, and finally, we suggest on which flaws and imperfection research has to focus its attention.

Background

Angiopoietins are part of a family that have a prominent role in vascular disease (Fagiani and Christofori 2013) and vasculature upregulation of many types of tumours (White et al. 2003). The most extensively studied angiopoietins are Ang1 and Ang2, especially for their involvement in cancer therapy. Ang2 is a compact protein with three domains, named A, B, and P. The P domain is the most divergent (Figure 1), both in sequence and in structure, among the fibrinogen homologs, and it is the site of ligand binding for most fibrinogen domain-containing proteins (Barton et al. 2005).

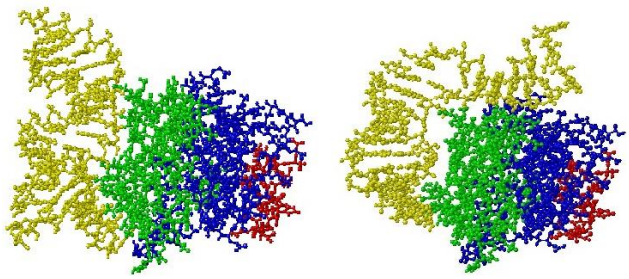

Figure 1. Hair (left) and Belt (right) aptamer-Ang2 conformers: in the Hair, the binding site of the aptamer (yellow) is primarily the P domain (green), while in the Belt, the binding site of the aptamer (yellow) is also in the B domain (blue).

Hu et al. (2015) selected and studied the affinity properties of a set of five sequences: two of them were natural anti-Ang2 and anti-Ang1 aptamers, while the other three were mutated sequences; the affinity for the target was evaluated by means of measurements with a surface plasmon resonance (SPR) biosensor. Conflicting results concerning the performances of the sequences were observed; in particular, the sequence with the best computational rank among the five considered (named Seq2_12_35) was one of the worst performing in experiments.

In (Cataldo et al. 2018) a procedure to cope with the problem of docking of ligands characterized by a large number of flexible freedom degrees was presented, by using as case-study the five aptamers proposed in (Hu et al. 2015). The 3D structure of the aptamers was obtained by using the free SimRNA software (Boniecki et al. 2015), docking was performed by means of rigid or flexible roto-translations in the free AutoDock Vina tool (Trott and Olson 2010). For scoring the complexes, a single and energetic quantity called "effective affinity" (EA) was proposed, putting together the docking energy provided by AutoDock Vina and the SimRNA energies.

In (Cataldo et al. 2019) a novel kind of scoring was addressed, starting from the same aptamers in (Hu et al. 2015). The study revealed a different type and abundance of conformers, which would best represent each aptamer-protein complex. The one, named Hair, mimics the binding with its natural protein target (Tie2), the other, named Belt, hangs the protein also far from the original binding domain (Figure 1). The results highlighted that: a. the effective affinity (Cataldo et al. 2018) seems not to be able to discriminate between these two types of conformers, because their values are quite similar; b. the Ang2-specific aptamer prefers Hair conformers; c. the Ang1-specific aptamer prefers Belt conformers; d. mutated sequences do not show a distinct preference. In other terms, it seems that the Hair conformer characterizes the high affinity complexes, while the Belt conformer characterizes the low affinity complexes.

Graph analysis

Starting from some seminal papers (Watts and Strogatz 1999; Albert and Barabási 2000), graph analysis has received increasing attention with applications to several fields of research, from physics to psychology. In fact, graph analysis is able to capture part of the complexity, which is inherent in most of natural phenomena. The inner idea is to explain multiscale-multiphase-many body phenomena by using interactions, thus in terms of networks.

Network has a topological structure given by the associated graph *G(N,L),* with *N* the node set and *L* the link set (Van Mieghem et al 2014). In proteins, the C_α atom of each amino acid is considered as a node, and two amino acids are considered nearest neighbors if the distance between their C_α atoms is less than an assigned threshold value. This distance or cut-off radius R_C is a free parameter; whose tuning produces a graph more or less connected (Alfinito et al. 2017). Once all the Euclidean distances between the couples of C_α atoms are calculated, a distance matrix is obtained; from the distance matrix, the graph description of the network is represented through its adjacency matrix *A* of size $N \times N$, with element:

$a_{i,j}$ = 1 (there is a link), if the distance between node *i* and *j* is less than the assigned R_C;

$a_{i,j}$ = 0 (there is no link), if the distance between node *i* and *j* is greater than the assigned R_C.

We assume that no self-loops (hence $a_{i,i} = 0$) and no overlapping links exist, i.e. there cannot be more than one link between $a_{i,j}$, therefore, we deal with a *simple graph* (Albert and Barabási 2000).

Among the various parameters that measure the graph characteristics, we point out on the following, giving a short definition.

Degree: the degree *k* is defined as the total number of network connections. The average of k_i over all *i* nodes is called the average degree *[k]* of the network. The spread in node degree is characterized by a distribution function *P(k),* which gives the probability that a randomly selected node has exactly *k* links (Wang 2002).

Assortative mixing and coefficient of assortativity (*r*): a network is said to show 'assortative mixing', if the high-degree nodes tend to be connected with other high-degree nodes, and 'disassortative' when the high-degree nodes tend to connect with low-degree nodes. It is the Pearson correlation coefficient of the degrees at either ends of a link and lies in the range $-1 \le r \le 1$; $r = 1$ means perfect assortativity, $r = -1$ means perfect disassortativity, $r = 0$ means no assortativity (random linking). If a network has perfect assortativity ($r = 1$), then all nodes connect only with nodes with the same degree.

Diameter and Shortest path length and average path length: the diameter *D* of a network is the maximal distance between any pair of its nodes. The distance L_{ij} between two nodes *i* and *j* is defined as the number of links along the shortest path connecting them. Then, the average path length L of the network is defined as the distance between two nodes, averaged over all pairs of nodes. L determines the effective size of a network, i.e. the typical separation of pairs of nodes (Wang 2002).

Clustering coefficient: clustering coefficient *C* can be defined as the average fraction of pairs of neighbors of a node, which are also neighbors of each other. Suppose that a node *i* in the network has k_i links, which connect it to k_i other nodes. These nodes are the neighbors of node *i*. Clearly, at most $k_i(k_i - 1)/2$ links can exist between them, and this occurs when every neighbour of node *i* is connected to every other neighbour of node *i*. The clustering coefficient *Ci* of node *i* is defined as the ratio between the number *Ei* of links that actually exist between these *ki* nodes and the total number $k_i(k_i - 1)/2$, namely

$$C_i = \frac{2E_i}{k_i(k_i - 1)}$$

The clustering coefficient *C* of the whole network is the average of *Ci* over all *i* (Wang 2002).

Largest eigenvalue (*lev*) depends on the highest degree in the graph. For any *k* regular graph *G* (a graph with *k* degree on all the vertices), the eigenvalue with the largest absolute value is *k*. Generally, *lev* increases if the graph contains

vertices of high degree, and decreases gradually from the graph with highest degree 6 to the one with highest degree 2 (Vishveshwara 2002).

Materials and Methods

In Table 1 are listed the five different RNA-aptamers employed for the research, they are the same sequences described in (Hu et al. 2015).

Name	Sequence
Seq1	AAAAAACUAGCCUCAU-CAGCUCAUGUGCCCCUC-CGCCUGGAUCAC
Seq16	AAAAAACUCGAACAUUUC-CACUAACCAACCAUA-CUAAAGCACCGC
Seq2_12_35	AAAAAUUAACCAUCAGAU-CAUGGCCCCUGCCCUCU-CAAGCACCAC
Seq15_12_35	AAAAAGAG-GACGAUGCCGACUAGCCU-CAUCAGCUCAUGUCCCCCUC
Seq15_15_38	AAAAAGAGGACGAUGCG-GAUUAGCCUCAUCAGCU-CAUGUGCCGCUC

Table 1. The five studied sequences, from (Hu et al., 2015). Seq1 is an Ang2-specific aptamer, Seq16 is an Ang1-specific aptamer, the other three are Ang2-mutated sequences.

In Table 2 are listed the percentage/number of Hair and Belt configurations took into account in this paper.

Name	Hair		Belt		
	%	n	%	n	Total n
Seq1	24	43	76	134	177
Seq16	44	103	56	133	236
Seq2_12_35	32	102	68	219	321
Seq15_12_35	35	55	65	102	157
Seq15_15_38	29	26	71	63	89

Table 2. The percentage/number (n) of the studied Hair and Belt configurations.

In the next, the results will be presented on violin plots; outliers, i.e. values more than 1.5 times the interquartile range, or approximately 3 standard deviations in a Gaussian distribution, have been dropped out. In general, we pay careful attention to the outliers, because they can signal skewed distributions rather than a statistical error in a unimodal and symmetric distribution. The RC value best representing our networks was 11.3 Å; this value was sufficiently large to have a connected network, but not so large to hide the node peculiarities (Alfinito et al. 2017).
Hereafter, the terms "network" and "graph" have to be considered synonyms.

Graph representation

In Figure 2 two typical networks for Hair and Belt conformers are drawn. Hair conformers seem to describe "golf-club" networks, in which aptamer is positioned above the Ang2 (dark grey zone), while Belt conformers describe a circular structure above the network protein (dark cyan zone). In both conformers, the average number of links is constant in all the sequences, reporting a value of 6150, for R_C = 11.3 Å.

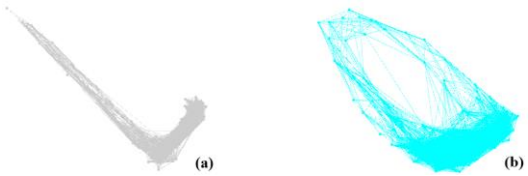

Figure 2. Networks for Hair (a) and Belt (b) conformer. The Hair conformer seems to describe a "golf-club" network, in which aptamer is positioned above the Ang2 (dark grey zone), while the Belt conformer describes a circular structure above the protein (dark cyan zone).

Assortativity

From Figure 3a, it is evident that the assortativity coefficient assumes a positive value and significantly greater than zero; it varies in the range [0.51- 0.55], showing a very slight increase, as a general trend, in the Hair conformers. Even if seemingly a distinct discrimination does not exist between the two conformations, Figure 3a clearly shows how the shape of the distribution for natural sequences (Seq1 and Seq16) is much more compact (Gaussian) than the mutated ones. These last sequences exhibit a less regular behavior, especially Seq15_12_35 and Seq15_15_38.

Hierarchy

From the findings, it is possible to state that the rank of the network is very high for low degree value; thus, even increasing the rank, the degree decreases exponentially. The average values are almost constant for all the sequences, in both

the conformations. Therefore, it seems that it is not possible to discriminate. Figure 3b shows this too weak fluctuation of hierarchy, even if Belt conformations linked to the natural sequences exhibit a more homogeneous shape of the distribution. Under certain aspects, this could be considered as an indicator of the quality of the in silico realization of the aptamer-Ang2 complex.

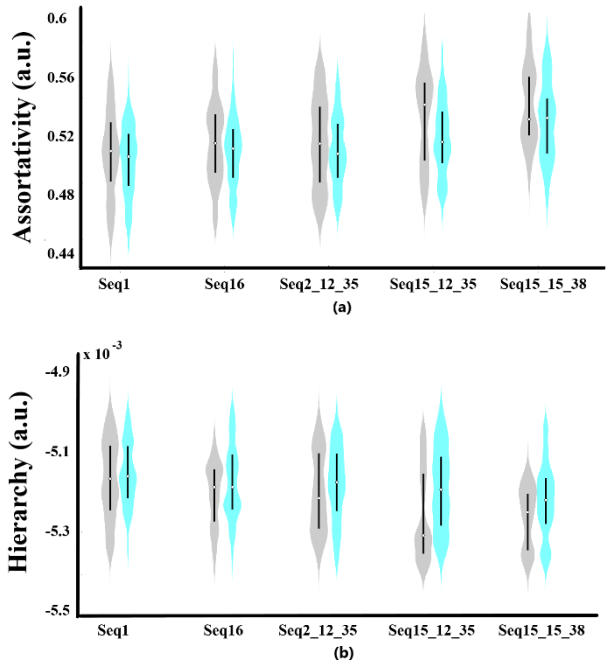

Figure 3. Assortativity (a) and hierarchy (b) coefficient distribution for the five aptamer-Ang2 complexes, gray for Hair and cyan for Belt.

Diameter

The diameter, intended as the maximum distance between two points of the network, could provide important information to discriminate between the two conformers. In particular, it could be hypothesized that the Belt conformers should have the highest values, since the aptamer, positioning itself around the protein, should increase the diameter of the network. However, this difference is not so evident (Figure 4a); the diameter turns out to be comparable for all the sequences, expressing for all the networks a *small-world* behaviour (Watts and Strogatz 1999).

Average length of the path

Figure 4b shows that the average path length in the Hair configurations is greater than the Belt ones, as expected. In fact, when aptamer surrounds protein, it is closer than when it is positioned above, namely in the Hair configuration.

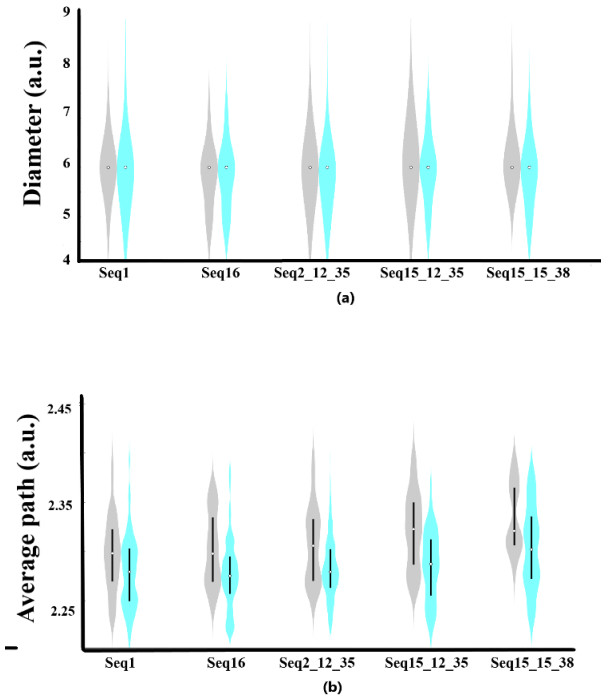

Figure 4. Diameter (a) and average length of the path (b) distribution for the five aptamer-Ang2 complexes, gray for Hair and cyan for Belt.

Average clustering coefficient

The average clustering coefficient is calculated as the average of all the coefficients of local clustering. Figure 5a shows that the Hair configurations values are higher than the Belt ones. In particular, the shape of the distribution points out appreciable variations for the mutant (Seq2_12_35, Seq15_12_35, and Seq15_15_38) compared to the natural (Seq1 and Seq16) sequences.

Maximum eigenvalue

The maximum eigenvalue of the Laplacian matrix was calculated according to the Perron-Fröbenius theorem. This eigenvalue has an associated eigenvector with no negative values for not oriented graphs, as those we examined. This is useful for centrality measures, in a sense that network with high centrality has many nodes with short contacts. This happens, as expected, for Belt conformers, as shown in Figure 5b.

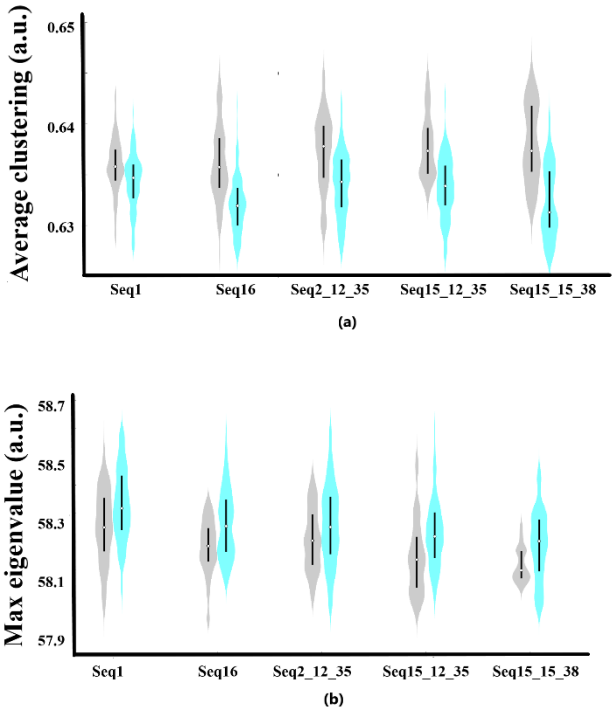

Figure 5. Average clustering coefficient (a) and maximum eigenvalue (b) distribution for the five aptamer-Ang2 complexes, gray for Hair and cyan for Belt.

Conclusions

Graph theory has been extensively applied in several fields of research, but applications to aptamer-protein complexes are still pioneering. The reasons reside in the fact that few structures are experimentally resolved, the results obtained by the application of the theory cannot be compared with a general, well-established model.

In (Cataldo et al. 2019) it has been observed that in silico aptamer-Angiopoietin complexes were arranged in two main conformers: Hair and Belt. Here, we study the same structures by using the concepts of graph theory with the aim to assess if the interpretation of networks could anticipate some characteristics, typical of those configurations.

From the graphs, differences between the two types of conformers are very clearly deduced, starting from the visual representations.

As for other topological parameters, the assortativity and hierarchy coefficients did not highlight a clear difference, but the shape of the distribution clearly reflects two different behaviours, able in appreciably discriminating the conformations. Discrimination becomes more evident in the average length of the path, the clustering coefficient and the maximum value of the eigenvalues of the adjacency matrix. In general, consistent differences are highlighted in the shape of the distribution for all the parameters, this is particularly true in the aptamer-Angiopoietin complexes derived from natural sequences (Seq1 and Seq16), compared to the mutated ones.

For the sake of completeness, another type of conformer (Figure 6) has been noted during the analysis of the networks. It is different from Hair or Belt and further investigations are needed in this regard.

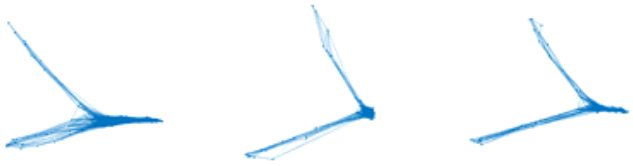

Figure 6. Examples of conformers, different from Hair or Belt configurations.

The achievement of these results can be considered a merit of the application of graph theory and, conversely, a precise indication of the fact that other efforts are required to perfect molecular simulation software, so that a description as close as possible to the real structure of the complexes can be reached.

We consider the proposed procedure effective in the screening process of the outcomes of different computational software, thus, it can be applied as a useful tool for increasing chances of success in designing high-specificity biosensors.

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Journal of InterDisciplinary Research Applied to Medicine
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2019), v. 2 i. 2, 35-41
ISSN 2532-7518
DOI 10.1285/i25327518v2i2p35
http://siba-ese.unisalento.it, © 2018 Università del Salento

Can an ECG help prevent sudden death in young people?

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Abstract
A wide spectrum of diseases or conditions (genetically based or acquired), in the presence of triggering elements, can lead to arrhythmic events, having sudden death as a common result. The substrate of fatal arrhythmia depends on age: over 35 years old it is mainly represented by the instability of coronary heart disease. Up to 35 years of age, the substrate consists mainly of electrical or structural alterations of the heart of a genetic nature the main of which are i) idiopathic hypertrophic cardiomyopathy ii) arrhythmogenic cardiomyopathy of the right ventricle iii) congenital syndrome of long QT iv) congenital syndrome of short QT;
v) Brugada syndrome. Each of these proarrhythmic genetic conditions has the prerogative of being able to be highlighted by an electrocardiogram: a simple and economical gesture that can lead directly to an important diagnosis, or at least make it suspected, addressing the subsequent evaluations. Formulating a diagnosis of this kind can minimize the risk of sudden death, through careful use of lifestyle advice, drugs, devices, procedures. It has been scientifically proved that identifying genetic conditions at risk of malignant arrhythmias in the athlete leads to a dramatic reduction in the risk of sudden death: on this basis, an electrocardiographic screening has become peremptory in many countries in order to be able to perform competitive sports (in Italy, for some years, also to be able to carry out non-competitive sports). Electrocardiographic screening should be considered for the entire youth population, regardless of participation in an organized sports activity program, as this can potentially mean saving young lives from sudden death.

Keywords: sudden death; electrocardiogram; hypertrophic cardiomyopathy; congenital long QT syndrome; Brugada's syndrome

Introduction

Sudden cardiac death (SCD) in young adults (<35 years) is an emotionally staggering event, not only for the victim's family but also for the community. Although the precise incidence of this catastrophic event is not exactly known around the world, in the USA it ranges between 0.8 and 8 deaths per 100,000/year (Vetter 2014, 688–97). As everywhere, even in Salento the SCD in young people is not a negligible event: the case of Lorenzo T., a 19-year-old male victim of SCD just as he had come out of a disco after a happy evening with friends, in which the autopsy examination revealed the presence of hypertrophic cardiomyopathy is perhaps the most sensational case, but it is only the tip of the iceberg. SCD is not a single disease, but the outcome of a wide spectrum of diseases or conditions in which a substrate (genetic or acquired) interacts with a series of physiological or pathophysiological factors (acute ischemia,

reperfusion, hyperadrenergic status, etc.) that modulate its arrhythmic propensity, in the presence of a triggering factor (Costantini 2019). This triad can lead to an intraventricular reentry (single or multiple), or result in post-potential triggered electrical activity, with the risk, in both cases, of producing a fatal arrhythmic accident. Such a paradigmatic sequence justifies most of the cases of sudden death, but the substrate is age-dependent: up to 35 years of age, the prevailing substrate is represented by electrical or structural alterations of the heart of genetic origin; above the age of 35, it is mostly represented by the consequences of a coronary heart disease (Costantini 2019). An early recognition of these arrhythmogenic conditions in the juvenile population could markedly reduce the risk of sudden death, as already evidenced in young athlete (Vetter 2014, 688–97). Below we will consider some of the characteristics related to the main hereditary proarrhythmic conditions.

Hypertrophic cardiomyopathy

It is a hereditary disease of the heart muscle, caused by mutations in the genes that encode sarcomere proteins (Pasquale et al. 2012, 10–17). The genetic abnormality results in a particular anatomical situation (myocardial hypertrophy, often asymmetric, sometimes monstrous; structural disorganization of the myofibrils, "disarray"; fibrosis) which determines a rather wide spectrum of functional and clinical alterations ranging from total asymptomaticity to myocardial ischemia, diastolic dysfunction, obstruction of left ventricular efflux, life-threatening arrhythmias and sudden death (Spirito, Quarta and Autore 2009, 1104–10). It is therefore a rather complex heart disease that has a high prevalence, estimated between 1/500 and 1/1000. The disease often remains undiagnosed. The phenotypic aspects include left ventricular hypertrophy, sometimes marked, often asymmetric, cavity of the left ventricle not dilated, conserved systolic function, dynamic obstruction in the left ventricular outflow tract (in 20% of cases), electrocardiographic changes, arrhythmias, sudden death (Spirito, Quarta and Autore 2009, 1104–10). The clinical course is extremely variable. Many patients have normal life spans, remaining completely asymptomatic. Others develop heart failure, others die suddenly, often at a young age and without any warning symptoms. Massive hypertrophy and disarray, together with fibrosis, constitute a favorable substrate for several arrhythmogenic mechanisms that can be favored by intercurrent modulating factors (autonomous nerve factors, emotional stress, acute myocardial ischemia, electrolyte disturbances, drugs), with arrhythmias of various kinds, up to malignant ventricular forms. Usually the disease is suspected for the presence of a pathological ECG, with QRS alterations (often Q of necrotic aspect) and ventricular repolarization alterations (negative T waves in anteriorior and/or lateral and/or inferior leads, figure1) , during a screening for sports activity or for family history of hypertrophic cardiomyopathy or less often for symptoms (Migliore et al. 2012, 529–38). The diagnosis is then confirmed by an echocardiogram. In a minority of cases, a clearly pathological ECG is not followed by the presence of left ventricular hypertrophy at the echocardiogram (Spirito, Quarta and Autore 2009, 1104–10). In such cases (phenotypically incomplete) cardiac nuclear magnetic resonance may help.

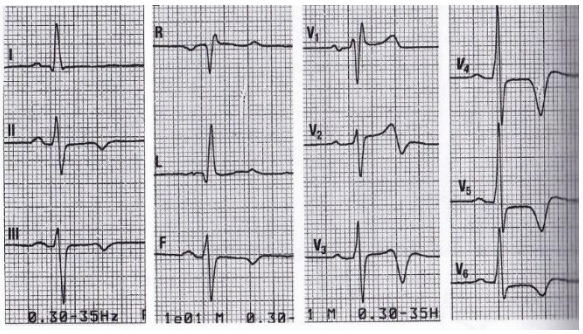

Figure 1. Classical ECG pattern in HCM

Arrhythmogenic Right Ventricular Cardiomyopathy/ Dysplasia (ARVD)

ARVD is a progressive disease of myocardial tissue clinically characterized by ventricular arrhythmias, heart failure, sudden death (Domenico Corrado and Thiene 2006, 1634–37). The distinctive character of the disease is the replacement of cardiomyocytes with fibro-adipose tissue with a particular structural and functional involvement of the right ventricle (but in more than half of the patients the left ventricle is also affected). In most cases it is a genetically determined inherited disorder with

autosomal dominant transmission caused by mutations in the genes encoding desmosomal proteins, plakoglobin, desmoplakina, plakofilina-2, desmoglein-2 and desmocollin-2. The disease has a variable prevalence from 1/1000 to 1/5000 subjects in the general population and represents one of the main causes of sudden death in athletes and young people. Clinical manifestations generally develop between the second and fourth decades of life and include palpitations, syncope, ventricular tachycardia, sudden death (D. Corrado et al. 2009, 1097–1103). The morphological progression of the disease can lead to right or biventricular heart failure. In basal ECG, abnormalities of various kinds are found in about 90% of cases, very useful both for making the diagnosis and for establishing the prognosis and the evolution of the disease (figure 2): P wave accentuated; prolonged PR tract; extension of the QRS duration; right bundle branch block of varying degrees; presence of epsilon wave (small positive low voltage deflection in the final part of the QRS or at the beginning of the ST segment in the right precordials: it expresses delayed activation of portions of the right ventricle and corresponds to the late recordable potentials with high resolution electrocardiography); negative T waves in the right precordial leads (D. Corrado et al. 2009, 1097–1103). The arrhythmic spectrum of the disease is variegated and ranges from ventricular, isolated or repetitive extrasystoles, sustained ventricular tachycardia, and ventricular fibrillation.

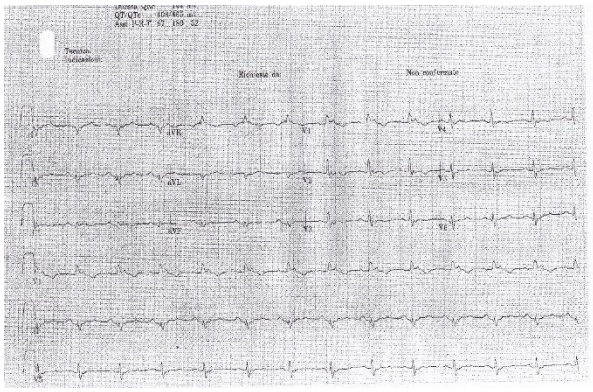

Figure 2. ECG of a subject with ARVD. Note the altered QRS, with right bundle branch block and probable epsilon wave in V1

Brugada's syndrome

It is a genetically based electrical disease that involves risk of sudden death (Brugada and Brugada 1992, 1391–96). The incidence of the condition is currently estimated around 5 cases per 10.000 (Antzelevitch et al. 2005, 429–40). Affected subjects have a defect in the gene that codes for the sodium channel: its function is depressed, leading to a transmural electrical inhomogeneity, which creates a voltage gradient between the epicardium and the endocardium. It is likely that the voltage gradient is due to an early repolarization in epicardial cells of the right ventricle: the genetic defect produces in these cells a deficit of sodium current during the phase 1 of the action potential, altering the balance between depolarizing and repolarizing forces, creating a strong predominance of the potassium repolarizing current (Ito). Since the subendocardial cells have a lower concentration of Ito channels, a clear difference in silhouette of the action potential between endocardium and epicardium takes place, producing voltage gradient, which favors the possibility of re-entry triggering in phase 2.
Classically, three different types of Brugada's patterns are derived from the basic ECG (Priori and Cerrone 2009, 1086–96):

- •Type 1: in this case it is evident a convex elevation of the ST tract (coved type) of at least 2 mm with evident J wave and negative T wave in V1 and / or V2 (figure 3);
- •Type 2: the ECG shows at V1 and / or V2 an elevation of the point J of at least 2 mm and an elevation of the ST segment of at least 1 mm and a positive or diphasic T wave;
- •Type3: there is an elevation of the point J of less than 2 mm and a saddle back elevation of ST of less than 1 mm with a positive T wave in V1 and/or V2.

Experts agree that only type 1 is diagnostic of syndrome (or pattern). Pattern 1, if not present in basal, can be disclosed by a pharmacological test, with flecainide or ajmaline, intravenously injected with great caution and under strict electrocardiographic and clinical control. Sometimes the typical ECG alteration in right precordial derivations is absent or just mentioned but appears or becomes more striking if the electrodes of the precordial right V1 and V2 are

moved higher (Priori and Cerrone 2009, 1086–96). The typical basal ECG aspect of the syndrome is not fixed, but it can suffer fluctuations (important to record ECG during fever). The sudden death is caused by a polymorphic ventricular arrhythmia and occurs especially during sleep or rest. Fever can be a factor of risk for malignant ventricular arrhythmias in affected subjects. The transmission of the condition is autosomal dominant with age penetrance and sex-dependent: clinical manifestations are more common in adult subjects and are 8 times more frequent in men than women. Ventricular fibrillation occurs at an average age of 41 ± 10 years, but can appear at any age, usually at rest or during sleep. Temperature, abuse of alcoholic beverages and large meals can reveal the ST segment elevation, with type 1 –appearance and predispose to the onset of malignant arrhythmias. Pharmacological treatments with drugs that produce an accentuation of the defect of the ion channels (such as sodium-blockers antiarrhythmics), may represent a major risk in affected patients (Priori and Cerrone 2009, 1086–96).

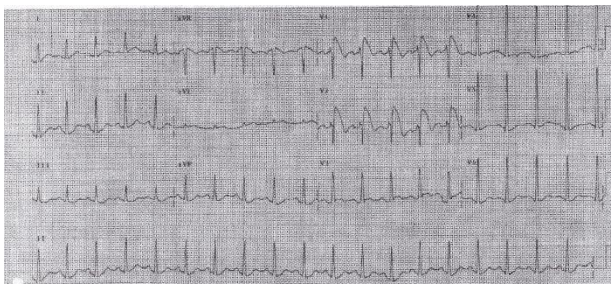

Figure 3. Classical Brugada's-Type 1 pattern

Congenital long QT syndrome (LQTS)

Genetically transmitted electrical disease, characterized by prolonged QT and high risk of malignant ventricular arrhythmias (torsade de pointes, which sometimes degenerates into ventricular fibrillation). Most arrhythmias arise in conditions of mental or physical stress. The beta-blocker therapy markedly reduces the mortality of this condition and therefore every effort is necessary for the diagnosis to be made in the affected persons (Schwartz, Periti and Malliani 1975, 378–90). The genes involved are numerous, and hence the multiplicity of the varieties already described , but the most frequent forms of LQTS are the first three varieties: the first two (LQTS 1 and LQTS 2) derive from a genetic defect of potassium channels with loss of function; variety 3 (LQTS 3) derives from a genetic defect of the sodium channel, with a gain in function (Schwartz and Crotti 2009, 1071–78).

PJ Schwartz proposed a very useful risk score for the cardiologist in diagnostic judgment (PJS score), which is based on the following elements: ECG and documented arrhythmias, medical history and family history (Schwartz and Crotti 2009, 1071–78). A PJS score ≥3.5 can be associated with a higher probability of disease presence. With a score between 1 to 3, the probability that the disease is present in the patient is intermediate, while a PJS score ≤1 can be associated with a low probability of LQTS. Often, a careful analysis of the ECG allows to differentiate between the various types of LQTS (at least among the main ones), therefore it is sometime possible to identify the genotype with a simple electrocardiogram (Schwartz and Crotti 2009, 1071–78). Thus, in type 1 a "homogeneous" elongation of the QT is classical, with evidence of very broad base T waves. In type 2 the presence of "notched" T in many derivations is characteristic (figure 4). In type 3 (the one that originates from an exalted sodium current and that responds less to beta-blocking therapy) the late manifestation of the T wave is characteristic: it occurs late, after a long ST segment often quite isoelectric. The existence of gene-specific triggers of arrhythmic events, different in the various forms of congenital long QT, is demonstrated (Schwartz and Crotti 2009, 1071–78). Thus, subjects with LQT 1 are at higher risk of arrhythmias during exercise (especially during swimming). Subjects with LQT 2 are very sensitive to loud noises, especially during sleep. Subjects with LQT 3 are more at risk during sleep or rest.

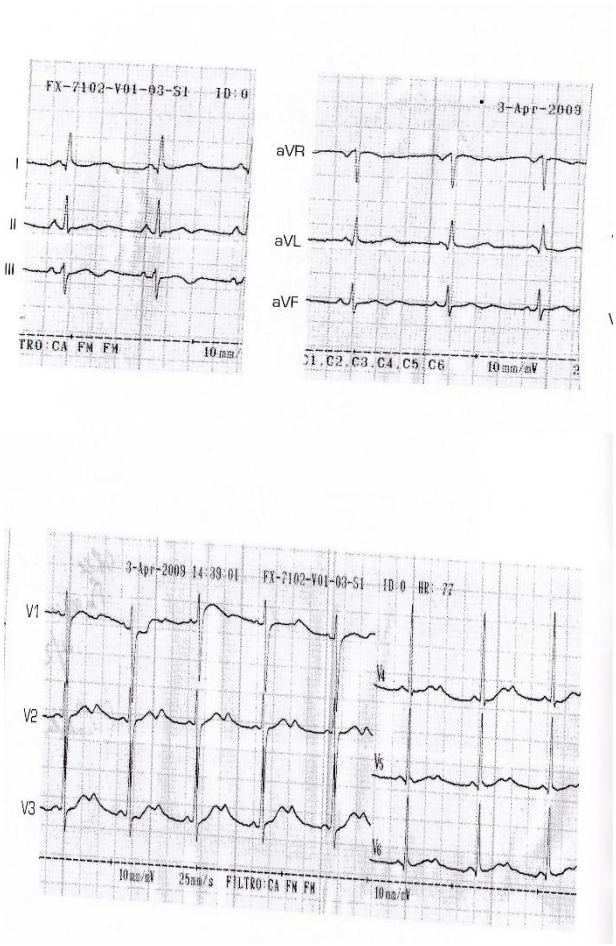

Figure 4. Type 2 congenital long QT syndrome

Short QT syndrome (SQTS)

Short QT syndrome is a genetically transmitted disease represented by the association between a very short QT to the ECG together with a propensity to develop atrial and ventricular tachyarrhythmias, in the absence of cardiac structural alterations (Gussak et al. 2000, 99–102). The mainstay of diagnosis of short QT syndrome is the 12-lead ECG: the diagnosis is made when duration of corrected QT is ≤340 ms (figure 5). Other features that may be seen on the ECG in short QT syndrome include tall, peaked T-waves and PR segment depression (Giustetto et al. 2006, 2440–47). The prevalence of SQTS in the population is likely around 0,1%. The disease appears to be associated with high lethality in all age groups, including children in early life. Criteria scientifically validated as independent risk factors for cardiac arrest are lacking, and therefore remains to be clearly defined what is the optimal primary prevention strategy in asymptomatic patients with this condition. No data are available to stratify the arrhythmic risk during competitive physical activity. Some reports show that quinidine is effective in prolonging the QTc interval and is likely to reduce arrhythmic events in these patients.

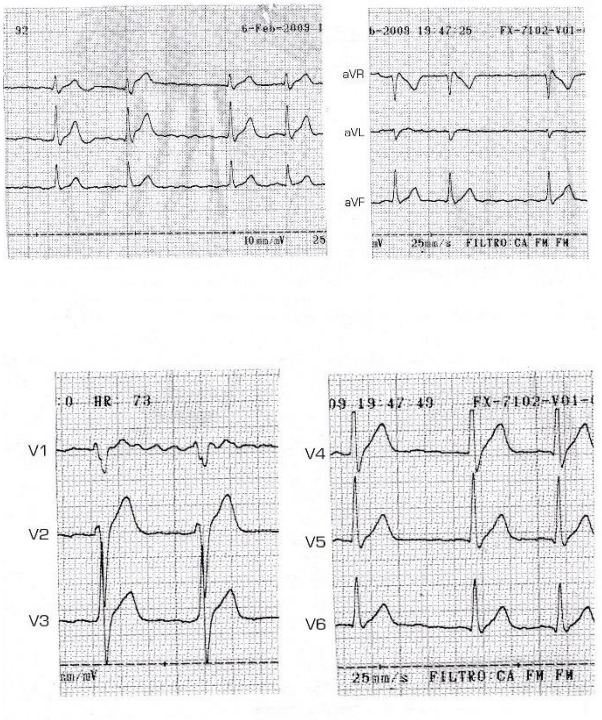

Figure 5. ECG of a subject with short QT and atrial fibrillation. Atrial arrhythmias are common in short QT syndrome.

All the conditions considered above have the prerogative of being able to be highlighted with an electrocardiogram: a simple and inexpensive gesture can therefore lead directly to the diagnosis or can make it suspect. Diagnostic assessment can minimize the risk of fatal events, through appropriate use of behavioral advice, drugs, devices, procedures. There is a scientific evidence that ECG screening identifies athletes who are harboring potentially serious arrhythmic events and that this strategy strongly reduces the incidence of SCD in competitive athlete (Domenico Corrado et al. 2003, 1959–63); for these reasons, ECG screening in athletes is endorsed by learned scientific and sporting organizations. Why then is a similar approach not extended to the entire youth population regardless of participation in an organized sports activity program? Something is indeed forgotten: although intense exercise in individuals

harboring quiescent cardiac pathology leads a 3-fold greater risk of SCD in elite athletes compared with non athletes, the majority of SCD in the young affect the general population because a lower incidence acts on a broader population (Domenico Corrado et al. 2003, 1959–63). In most countries, young people are not offered cardiac screening unless they are engaged in top-level competitions, but this is in contrast, both ethically and legally, with the fundamental principle of equality of all individuals. Perhaps, it is time to reflect on the ECG screening of the entire youth population, not only of the athletes. In the 1960s the World Health Organization (WHO) adopted scientific criteria for the evaluation of health screening programs (A.a.v.v. 1968, 318).

According to these criteria, a screening program is justified if:

1) the morbid condition to be identified is relevant from a public health point of view;
2) there is a proven efficacy test for the early identification of the morbid condition, to allow early treatment;
3) there are effective therapeutic measures for the morbid condition if diagnosed at an early stage;
4) there is evidence that such therapies, if started in the presymptomatic phase, improves the clinical course and the prognosis of the morbid condition.

We believe that all these criteria are met by an electrocardiographic screening in youth age and that also the cost-effective ratio would be advantageous (Domenico Corrado et al. 2011).

But a few important issues arise:

1) What about false negatives? Some congenital conditions at risk of sudden death are not accompanied by alterations in the basic electrocardiogram. Among these, we must mention the Catecholaminergic Polymorphic Ventricular Tachycardia, the congenital anomalies of coronary origin, the aortopathies. It should also be considered that even in the ECG-evident proarrhythmic conditions the diagnosis can escape due to the dynamic character of the ECG-changes, which may be elusive at the time of screening (Priori and Cerrone 2009, 1086–96; Schwartz and Crotti 2009, 1071–78).

2) What about false positives? The ECG is dynamic by nature: up to 7 years, T waves in the right precordials are physiologically negative; successively, they gradually positivize. When the T waves remain negative beyond V1 after the age of 14, is it necessary to question whether one is dealing with a juvenile pattern or ARVD (18). Furthermore, regular and long-term intensive exercise is associated with several electrical manifestations that reflect enlarged cardiac camber size and increase vagal tone (Chandra et al. 2014, 2028–34). To facilitate the interpretation of the ECG in athlete, the European Society of Cardiology has produced guidelines able to differentiate the normal ECG scheme belonging to the trained subject, from the one relative to a subject with an underlying heart disease, although a certain overlap can still be observed in some cases (Chandra et al. 2014, 2028–34).

In addition to these, other issues of non-negligible importance emerge, such as the amount of subjects considered positive at screening, who need further investigations or eventually therapeutic measures and follow-up , with relative costs and assumption of responsibility by the health system and operators. Last but not least, there is a subtle question regarding the psychological impact of screening: discovering an arrhythmic condition certainly has markedly positive aspects, but we should ask ourselves what psychological impact it could have on an asymptomatic individual and his family, learning from an unknown doctor that his heart is at risk of serious arrhythmias.

In conclusions, prevention of SCD in young people, athletes and not, remains a high priority for the medical community.

We have the task of overcoming the difficulties and defining the most appropriate strategies to reach such an ambitious goal.

Disclosure

The authors declare that do not have a conflict of interest and that do not have a financial relationship with any commercial entity that has an interest in the subject of this manuscript.

Contributors

All authors participated to review. All authors were involved in writing and revising the article prior to submission.

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Journal of InterDisciplinary Research Applied to Medicine
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2019), v. 2 i. 2, 43-52
ISSN 2532-7518
DOI 10.1285/i25327518v2i2p43
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Intrapartum ultrasound during prolonged second stage of labor: a diagnostic tool suggested for operative delivery to reduce complications.

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Abstract
Diagnosis and management of prolonged second stage of labor and its complications is difficult and often poses a dilemma to the treating obstetrician regarding timing and type of intervention. Nowadays, the diagnosis of dystocic prolonged second stage of labor is largely based on digital evaluation of cervical dilatation and fetal head station and position, resulting inaccurate and subjective. Moreover, the problem of timing of delivery for nulliparа during dystocic labor and labor analgesia is clinically unsolved, as well as questioned since many years. Thus, labor management is largely based upon clinical and not instrumental findings. Women in dystocic labor require, often, operative delivery, after many hours of pain during labor. Accurate assessment of fetal head position and station is crucial in clinical decision-making during the second stage of labor and the fetal station was misinterpreted as lower than it really was in 15–22% of cases. Misdiagnosis or failure to correctly identify the fetal head position and station is one of the causes of failed instrumental delivery and subsequently of higher rate of neonatal morbidity. Intrapartum ultrasound also distinguishes patients destined for spontaneous vaginal delivery from those to submit to operative delivery. The intrapartum US is an adjunctive tool for labor ward obstetricians in the management of prolonged second stage and dystocia. It is a more objective and reliable tool than digital examination, and may give the obstetrician a more accurate perspective before making crucial clinical decisions regarding the chances of a successful vaginal delivery, and may lower the rate of failed instrumental delivery and its associated morbidity.

Keywords: Intrapartum ultrasound, prolonged second stage of labor, operative delivery, obstructed labor, dystocia, vacuum extractor, cesarean section.

Introduction

The philosophy of labor is based on two important queries: when and how to deliver. The answer to these two questions seems apparently simple but, on the contrary, it is extremely complex and abstruse. On the one hand there is the pregnant, full of anxiety and uncertainty about what will happen, on the other there is the fetus that must pass through the uterus that pushes it, the birth canal. In between there are dozens and dozens of variables and risk factors, for a sudden and unexpected modification of labor, while staying cognizant of monitoring safety and preventing harm.

The second stage of labor begins, basing on FIGO guidelines, from full dilatation of the cervix up to the birth of the singleton baby or the last baby in a multiple pregnancy (FIGO Safe Motherhood and Newborn Health (SMNH) Committee 2012, 111–16). At the start of the second stage, the fetal presenting part may or may not be fully engaged (meaning that the widest diameter has passed through the pelvic brim), and the woman may or may not have the urge to push (FIGO Safe Motherhood and

Newborn Health (SMNH) Committee 2012, 111–16)

Second stage may get unduly prolonged because of cephalopelvic disproportion (CPD), abnormal fetal position, and poor expulsive efforts resulting from conduction analgesia, sedation or maternal exhaustion. Many clinical factors can influence the progress of the second stage of labor.

These factors include maternal characteristics, such as age, parity, the size and shape of the pelvis, height and weight, uterine contractile forces, soft tissue resistance, expulsion effort, as well as presence of medical/obstetric conditions, including hypertensive disorders or pregestational/gestational diabetes mellitus. Fetal characteristics include birth weight, fetal occiput position/degree of flexion, and station at complete cervical dilation (Cheng and Caughey 2017, 547–66)

Prolonged second stage of labor is generally associated with several maternal and perinatal complications, including: increased operative vaginal delivery (OVD), cesarean section (CS), third- and fourth-degree perineal tear, cervical injury (with increased risk of preterm delivery in the subsequent pregnancy), post-partum hemorrhage (PPH) and chorioamnionitis. Neonatal complications include low 5-minute APGAR score, admission to the Neonatal Intensive Care Unit, birth trauma and birth depression (Cheng and Caughey 2017, 547–66; FIGO Safe Motherhood and Newborn Health (SMNH) Committee 2012, 111–16)

In management of a second stage of labor, there is a large evidence that digital obstetric examination does not provide an accurate assessment of the descend and position of the fetal head during the first and the second stage of labor (Malvasi et al. 2014, 520–26).

Investigations showed that digital obstetric examination during labor and delivery frequently fails to identify the correct fetal position in a high proportion of cases and ultrasonography in labor may play an important role in labor and delivery management (Sherer et al. 2002, 258–63; Sherer et al. 2002, 264–68)

Recent studies using intrapartum ultrasound (IU) have described objective measures of progression of the fetal head during labor, with a reduced error in diagnosis of fetal head position and progression (Malvasi et al. 2016, 2408–13)

Prolonged second stage may be managed by oxytocin augmentation, instrumental delivery or caesarean section and the safe use of vacuum extractor (VE) and forceps during OVD assumed the use of IU for the correct determination of the fetal head position and appropriate application of the instrument (Gustapane, Malvasi and Tinelli 2018, 540–41).

The use of IU is of fundamental importance for a safe OVD and can help in the prediction of whether a vaginal delivery would be successful, since scientific evidences suggested that IU may play an important role in the prediction of the time of onset and the progress of labor (Chor, Poon and Leung 2019, 31–37; Barak et al. 2018, 9–14; Choi et al. 2016, 3988–92)

The modern obstetric management of second stage is an ongoing challenge to reduce rates of emergency cesarean deliveries and to avoid adverse maternal and neonatal outcomes, since spontaneous parts are reduced and maternal and fetal complications are increased.

Thus, we performed a literature analysis to assess the impact of IU on management of prolonged second stage of labor, to assess its utility for operative delivery and if it can reduce complications.

Literature analysis

The safe balance between maternal and neonatal benefits and risks during the prolonged second stage of labor has been hindered by a lack of high-quality, prospective studies. The length of the second stage of labor was primarily defined as the duration between complete cervical dilation and delivery of the fetus. According to American College of Obstetricians and Gynecologists (ACOG) Practice Bulletin No. 49 on Dystocia and Augmentation of Labor, a prolonged second stage was defined as more than 2 hours without epidural or 3 hours with epidural analgesia in nulliparous women, and 1 hour without, or 2 hours with epidural analgesia for multiparous women (American College of Obstetrics and Gynecology Committee on Practice Bulletins-Obstetrics 2003, 1445–54) The additional hour allotted for labor with epidural anesthesia appeared to be based on the mean effect of epidural (Albers 1999, 114–19; Albers, Schiff and Gorwoda 1996, 355–59)

Subsequently, there were other literature data about the duration of the second stage of labor; recent recommendations often include longer durations in some cases, that is, that management is individualized depending on progress of labor, epidural analgesia, fetal position and interventions (Cheng and Caughey 2017, 547–66)
An obstetric care consensus in 2014, on the safe prevention of primary CS, the ACOG and the Society for Maternal Fetal Medicine (SMFM) allow an additional 1 hour of extended pushing in the second stage of labor for nulliparous and multiparous women before diagnosing second-stage arrest (Caughey et al. 2014, 179–93)
The extended second-stage labor represents a promising approach for balancing maternal and fetal risks, while working to reduce the rate of primary CS; nevertheless, the literature data are not yet sufficient to demonstrate the benefit of reducing CSs compared to maternal and fetal complications that may arise after a prolonged second stage of labor.
We searched on PubMed/Medline, Scopus, Google Scholar, EMBASE, the Cochrane Database, and a previous review the following key words: prolonged second stage of labor, intrapartum ultrasound, dystocia, operative delivery, vacuum extractor, cesarean sections and complications, to identify relevant articles published from 2000 and 2019 and to find the conclusions to our queries.

Intrapartum ultrasound in labor

The literature analysis has amply demonstrated that, in the case of prolonged labor, the IU is much more reliable than the vaginal visit in the obstetric diagnosis of situation, position and fetal progression.
Studies largely demonstrated the major diagnostic accuracy of IU to diagnose, during labor, the fetal head position (Sherer et al. 2002, 258–63; Sherer et al. 2002, 264–68), station (Dupuis et al. 2005, 193–97) and internal rotation (Ghi et al. 2009, 331–36; Malvasi et al. 2016, 2408–13) in the maternal pelvis, in comparison of traditional digital vaginal examination.
Similarly, different trials report the superior diagnostic value of IU in the malpositions (Bellussi et al. 2017, 633–41) and malrotations, (Simkin 2010, 61–71) during dystocic labor and delivery, especially in Occiput Posterior Position (OPP) (Bellussi et al. 2017, 633–41) and asynclitism (Simkin 2010, 61–71).
Moreover, the use of US did not have any negative impact on neonatal morbidity and mortality, basing on literature data.

Intrapartum ultrasound in prolonged second stage of labor

Abnormal descent pattern leads to prolongation of the second stage of labor. This abnormal descent is of two types: protracted descent and arrest of descent. Protracted descent is defined as descent of presenting part by less than 2cm per hour for multiparous women, and less than 1cm per hour for primiparous women. The arrest of descent is defined as no descent of the presenting part for more than one hour. Both may be an indicator of obstructed labor that needs an accurate IU diagnosis and a prompt intervention by OVD or CS. Nevertheless, prolonged attempts at VE are associated with neonatal morbidity and maternal trauma, especially so if the procedure is unsuccessful and an urgent CS is performed.
Moreover, important potential complications arising in the prolonged second stage of labor are fetal hypoxia and acidemia leading to "birth asphyxia," failure of the presenting part to rotate or descend appropriately leading to obstructed labor, and worsening or new manifestations of maternal hypertension leading to eclampsia (Sandström et al. 2017, 236–42).
Maternal complications after a prolonged second stage of labor are: infections, urinary retention, hematomas or ruptured sutures, especially in the early postpartum period; pregnants with pre-existing cardiac disease or severe anemia may be at risk of heart failure during the prolonged second stage, owing to the additional circulatory demands of active pushing (Stephansson et al. 2016, 608–16)
When patients have a delay or prolongation of the second stage, a prompt and thorough clinical assessment by IU should be recommended, to rule out full bladder, malposition or/and malpresentation of the fetal head, apart the inadequate uterine activity, poor pushing effort, all signs of obstructed labor.

Thus, the IU can accurately determine fetal head position, station and progression in delivery canal, during the second stage of labor, and it can be of great help in the management of prolonged second stage of labor.
An urgent CS during prolonged second stage of labor, "especially for a deeply engaged head" can be a nightmare for all obstetricians, since it can lead complications including: bladder injury while opening up of abdomen, difficulty in delivery of head, lateral extension of the angle causing broad ligament hematoma, tear of lower uterine segment (LUS) & downward extension of scar that may involve bladder, difficulty in tracing retracted LUS after surgery for which one may take a stay suture earlier, accidental incision over vagina, PPH, puerperal infection and later fistula formation and pelvic organ prolapse (POP).
Delivery of a deeply impacted head may pose a problem even during caesarean section.

Intrapartum ultrasound and operative delivery

Generally, misdiagnosis or failure to correctly identify the fetal head position and station is one of the causes of failed instrumental delivery and subsequently of higher rate of neonatal morbidity (Ben-Haroush et al. 2007, 308.e1-308.e5; Murphy et al. 2001, 1203–7; Hiraizumi et al. 2012, 280–83).
The VE and forceps are useful tools for conduction of vaginal delivery in prolonged second stage, to shorten and reduce the effects of the second stage of labor on maternal/fetal conditions. Literature data report a failure rate of 4%–8% for instrumental delivery, especially among women with risk factors such as obesity, fetal occipital-posterior position, and mid-cavity delivery (Bhide et al. 2007, 541–45; Aiken et al. 2014, 796–803; Murphy et al. 2001, 1203–7)
The US has been suggested as a more objective and reliable tool than digital vaginal examination for assessing fetal head position and station as well as in predicting the success of labor (Ghi et al. 2018, 128–39)
Barak et al (Barak et al. 2018, 9–14) evaluated the impact of IU on VE attempts. They demonstrated that among women who also had an intrapartum US as part of the clinical decision-making process, during the second stage of labor, there was a trend toward a lower rate of failed VE (although not reaching a statistical significance), with lower rate of CS, higher rate of vaginal deliveries, and without significant differences in neonatal outcome. Authors reported also that in the "+US" group, the CS rate was lower than in the "no-US" group.
In addition, Duckelmann et (Dückelmann et al. 2012, 484–88) evaluated the impact of IU on decision making for VE application, in a cohort of women with a prolonged second stage of labor; authors showed that by using intrapartum US, they were able to lower the CS rate without increasing maternal and neonatal morbidity. This study concluded that the use of intrapartum US can also lower the rate of failed VE attempts.
Sainz et al (Sainz et al. 2016, 1348–52) evaluated the predictive capacity of intrapartum transperineal ultrasound (ITU) in prolonged second stage of labor, to predict cases of failure in fetal extraction in operative deliveries by VE. They evaluated the following IU parameters: Angle of Progression (AoP), Progression Distance (PD) and head direction (HD). In the transverse plane, midline angle (MLA) and head-perineum distance (HPD) were assessed. The VEs were classified as easy (three or less vacuum pulls), difficult (more than three vacuum pulls) or impossible (delivery completed by cesarean section or CS). In the results, authors observed that the presence of an AoP with pushing <105°, a PD <25 mm, a "head-down" direction and a >45° MLA are very unfavorable ITU parameters which can be used to identify cases of high risk of fetal extraction failure in vacuum-assisted deliveries. Thus, ITU can help differentiate easy (3 pulls or less), hard (more than 3 pulls), or impossible (CS was needed) VE trials and that it is possible to identify high risk cases for failed VE by using some TPUS parameters.
Chan et al (Chan et al. 2019, 192–98) evaluated patients in prolonged second stage of labor, measuring the AoP by ITU before, indicating an instrumental delivery or CS. Authors concluded that AoP predicted approximately 80% of successful OVD performed for prolonged second stage of labor. This study' observation was not surprising given that the AoP is known to widen as the fetal head descends along the birth canal, suggesting that a lowered fetal head

position in the pelvis might have favored vaginal delivery. The study also found that median AoP during contraction with pushing was 20°–30° wider than median AoP at rest, which implied the presence of fetal head descent with maternal pushing.
Several studies have suggested that an AoP of 120° was associated with successful vaginal delivery (Sainz et al. 2016, 1348–52; Chan et al. 2019, 192–98; Barbera et al. 2009, 313–19; Kalache et al. 2009, 326–30; Sainz et al. 2015, 2041–47) but other studies suggested that AoP cutoff values of 105°–145.5° were associated with difficult or failed instrumental delivery (Ghi et al. 2013, 430–35; Cuerva et al. 2014, 687–92; Bultez et al. 2016, 86–91)

Gilboa et al (Gilboa et al. 2015, 399–404) evaluated, in a prospective study, different sonographic methods for the prediction of the difficulty and the success of OVD in pregnants with prolonged second stage of delivery with cephalic presentation. The investigated parameters were the following: head station, passage of the biparietal diameter (BPD) of the infrapubic line (IPL), percentage of head after the IPL, head circumference after IPL were all correlated with the difficulty of OVD. When the distance between the widest diameter of the head and the IPL is <1.2cm, there is a 90% probability of success of OVD. When that distance is >3.3cm, there is 90% probability of cesarean section. When the percentage of head beyond the IPL was >54%, there was 90% probability of successful OVD.
Authors concluded that ITU was useful in the prediction of the difficulty and the success of OVD. The higher the extent of head that passed the IPL, the less difficult the OVD and the greater the success rate of the OVD.
Kahrs et al (Kahrs et al. 2017, 69.e1-69.e10) evaluated, in a prospective cohort investigation on 222 pregnants, if ultrasound measurements of fetal position and station can predict duration of VE, mode of delivery, and fetal outcome in nulliparous women with prolonged second stage of labor.
The duration of VE was shorter in women with HPD ≤ 25 mm (log rank test <0.01). The estimated median duration in women with HPD ≤ 25 mm was 6.0 (95% confidence interval, 5.2-6.8) minutes vs 8.0 (95% confidence interval, 7.1-8.9) minutes in women with HPD >25 mm. The HPD was associated with spontaneous delivery with area under the curve 83% (95% confidence interval, 77-89%) and associated with CS with area under the curve 83% (95% confidence interval, 74-92%). In women with HPD ≤35 mm, 7/181 (3.9%) were delivered by CS vs 9/41 (22.0%) in women with HPD >35 mm (P <.01). Ultrasound-assessed position was occiput anterior in 73%. Only 3/138 (2.2%) fetuses in occiput anterior position and HPD ≤35 mm vs 6/17 (35.3%) with non-occiput anterior position and HPD >35 mm was delivered by CS. Umbilical cord arterial pH <7.10 occurred in 2/144 (1.4%) women with head-perineum distance ≤35 mm compared to 8/40 (20.0%) with HPD >35 mm (P < .01). They concluded that IU has the potential to predict labor outcome in women with prolonged second stage of labor.
Zipori et al (Zipori et al. 2019, 191.e1-191.e7) recently changed their approach to labor dystocia, as recommended by ACOG/SMFM (Caughey et al. 2014, 179–93), extending the length of prolonged second stage of labor; they significantly decreased the primary CS rate, in both nulliparous and multiparous women. However, this practice of extending the second stage of labor was associated with a small rise in OVD among nulliparous women, as well as with increases in other immediate maternal complications, specifically, higher rates of PPH and of third- or fourth-degree perineal lacerations. In assessing the neonatal complications, they noticed a higher rate of low umbilical artery cord pH in period II, but the early neurological outcome did not change. Authors concluded that in a prolonged second stage of labor, a CS can be done in all cases of doubt in order to prevent failed OVD, but a reduced rate of failed VE/forceps will be accompanied by an increased emergent CS rate. Thus, the benefits of safe prevention of primary CS, by extending the duration allowed for the second stage of labor, must be weighed against the potential adverse maternal and neonatal outcomes.
Muraca et al (Muraca et al. 2017, E764–72) investigated the effect OVD at mid-pelvis to reduce the CS rate, trying to quantify severe perinatal and maternal morbidity and mortality associated with attempted mid-pelvic OVD on more of 180000 pregnants. Among women with dystocia and prolonged second stage of

labor, mid-pelvic OVD was associated with higher rates of severe perinatal morbidity and mortality compared with CSs, especially with higher rates of severe birth trauma. Rates of severe maternal morbidity and mortality were not significantly different after OVD, although rates of obstetric trauma were higher.
Authors concluded that mid-pelvic OVD was associated with higher rates of severe birth trauma and obstetric trauma, whereas overall rates of severe perinatal and maternal morbidity and mortality vary by indication and operative instruments.

Conclusions

During a prolonged second stage of labor, assessments of the balance of risks and benefits between mid-pelvic OVD and CS have tended to favor the latter option in recent decades to reduce maternal neonatal complications (Sandström et al. 2017, 236–42; Shmueli et al. 2017, 886–89; Altman et al. 2015, 1209–15; Salman et al. 2017, 1145–50; Stephansson et al. 2016, 608–16) and this has contributed to a rising rate of CS worldwide (Zizza et al. 2011, 161–73; Boerma et al. 2018, 1341–48)
Diagnosing and managing of a prolonged second stage of labor is challenging, and prolonged second stage diagnoses will affect 10% to 14% of nulliparous and 3% to 3.5% of multiparous women (Cheng and Caughey 2015, 227–40)
Currently, the decision to perform OVD is traditionally based on subjective assessment by digital vaginal examination and clinical expertise and there is currently no method of objectively quantifying the likelihood of successful delivery.
The routine uses of IU or ITU should be encouraged during labor and in delivery room since there is large scientific evidence that digital obstetric examination either for the determination of fetal head position during labor or in the descent of the head in the birth canal is not accurate and IU is effective and feasible for a correct diagnosis (Tinelli, Di Renzo and Malvasi 2015, 310–11; Malvasi et al. 2015, 1890–94; Gustapane, Malvasi and Tinelli 2018, 540–41)
Moreover, for the successful and safe use of OVD, the correct determination of the fetal head position and appropriate application of the instrument by IU or ITU can reduce also the medical legal liability for VE failure or maternal and neonatal complications (Malvasi et al. 2018, 1108–9; Eggermont 2015, 87–95)
In fact, the IU demonstrated, also in such case, its great utility and precision in indicate, indirectly, the correct placement of the vacuum cup on the flexing point and placement of the forceps blades parallel to the sagittal suture.
Both features are associated with high success rate and reduction in maternal and fetal morbidity, since OVD is an integral part of obstetric care and is indicated for prolonged second stage of labor or fetal compromise or to shorten the second stage of labor for maternal indications.

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Journal of InterDisciplinary Research Applied to Medicine
JDREAM
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2019), v. 2 i. 2, 53-59
ISSN 2532-7518
DOI 10.1285/i25327518v2i2p53
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Biopolytics and Bioeconomics in health: the paradigm of risk in informed consent and defensive medicine

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Abstract
At the beginning, it was the French philosopher Michel Foucault who explicitly defined medicine as a matter of biopolitics, making evident the role that, in his opinion, medical knowledge assumed in certain strategies of power. Starting from this assumption, the bioeconomic paradigm has been embodied in a form of governmentality of human behaviors, where the individuals are first and foremost considered biological living units. The biopolitical and bio-economic paradigm must not be considered as a space of absence of power, but a place where power leads to obedience by activating alternative devices, acting on population wishes and needs. These dynamics have slowly modified and even subverted the relationship between doctor and patient, determining the default of the paternalistic relationship and the strengthening of defensive medicine.

Keywords: biopolytics, bioeconomics, health, defensive medicine

Biopolitics and bioeconomy

The French philosopher Michel Foucault, in the second half of the last century, proposed a fructuous reflection focused on the concepts of biopower and biopolitics, whose interpretation, however, is not univocal; it is useful, therefore, to consider, in the first instance, some meanings that the terms "biopower" and "biopolitics" assume in the huge production of the author.
In 1974, Foucault spoke for the first time about the concept of biopolitics during the conference held in Rio de Janeiro about " The birth of social medicine". On this occasion two fundamental data emerge: the first, concerning the connection between biopolitics and capitalism, able to determine the possibility of implementing a control on individuals, not only by consciousness and ideology, but also through the body.
The second, because of the first, is the importance that the body assumes as a biopolitical reality and medicine as a biopolitical strategy (Foucault, 1997, 222).
The real focus of Foucaultian researches, however, is not the living body, but rather its constitution as a scientific object, as the specific knowledge indispensable for the exercise of a certain power. For this reason, Foucault's attention is often turned to all the published and inedited scientific speeches on the body.
The initial reference of biopolitics to the body and medicine changes and expands a couple of years later, when he explains how the life power has developed in two directions, one focused on the body-machine, the other on the body-species. In the first case, it is an anatomical-political of the human body. It is materialized through processes of discipline and supported by mechanisms of power, both focused on the body to strengthen it, but also to make it "docile" and to incorporate it into effective and economical control systems.
In the second case, Foucault speaks specifically of biopolitics of the population, as the set of interventions and regulatory controls that, since the mid XVIII century, have addressed the set of living bodies constituting the population. Also, in this perspective, the binomial constituted

by knowledge and power assumes a fundamental role. The affirmation of specific fields of knowledge, which not only include medical knowledge, but also demography, statistics and political economy, determines the possibility of managing the dynamics concerning health, hygiene, food, sexuality and all the biological processes shared by living beings, as birth, proliferation, mortality, etc.

Foucault precise that until the XVIII century, such management of the bodies were performed according to the directions mentioned above in a distinct way. Together they inaugurated bio-power era, which the author considers as an essential element for the development of capitalism, because of the controlled introduction of the bodies in the production chain and the adaptation of population phenomena to economic processes (Foucault, 1978, 123-124). The State has therefore adopted an economic rationality, determining the state transposition of "oikonomia" (the administration of the house), making it as a specific mode of intervention in the public sphere (Esposito, 2015, 19-20).

Biopower, moreover, has shifted attention to the concept of norm, which has also begun to make its way in the legal context. Although it does not replace the law (i.e. the legal norm connected to power in the classical sense), it has undoubtedly led the law to function as a norm. It can be deduced from the fact that the juridical institution has increasingly integrated itself with other apparatuses, having medical and administrative regulatory functions. Unlike the law, which differentiates individuals based on what they do, playing on the contraposition between lawful and unlawful and, consequently, prohibiting or condemning, the norm usually identifies and differentiates both individuals and populations based on what they are, focusing on the normal-pathological duality.

What is outlined is, finally, a new technology of power, which Foucault sees fully realized in the framework of the so-called liberal "governmentality". Because of the mentioned concepts, it appears not attributable to the simple juridical analysis of sovereignty. Moreover, biopower requires the contribution of the economic dimension. The object of this governmentality is the sum of singular and collective living beings, anatomical bodies and biological bodies. The political rationality is in some measure forced to treat "omnes et singulatim", producing at the same time individualizing and totalizing effects (Foucault, 2001, 145-146).

Normalization and medicalization

In the medical field, the governmental process called "medicalization of society", characterized by "a generalized medical conscience", was implemented by the task of controlling either individual life, through the identification and treatment of diseases, or collective life, in order to define and implement specific health parameters. (Sorrentino, 2008, 108). In this context also hygiene represents, besides medicine, a specific field of knowledge within a structured regime of health of the populations. It was based on the analysis of the habitats (cities, districts and houses) and on the rates of morbidity and mortality of the inhabitants, justifying authoritarian medical interventions in all those places at risk for possible diseases and epidemics (Foucault, 1997, 195-196).

The importance of medicine, not only methodologically but also ontologically, in the constitution of human sciences has thus emerged. It was the possibility for the individual to be, at the same time, subject and object of his own knowledge (Foucault, 2005, XVII).

Until the middle of the twentieth century, however, the role of medicine connected to the problem of health, undoubtedly fundamental, was seen from an essentially nationalist perspective. It was particularly aimed at ensuring health, to preserve the national physical force, the workforce, production capacity and military strength.

For this reason, Foucault considered 1942 a date with a strong symbolic value. In that year the Beveridge plan was drawn up, showing, unlike what had happened in the past, that the phenomenon of the consolidation of medicine was connected for the first time to a right to health. In addition, even though it was made explicit in a world context in which paradoxically millions of human lives were suppressed, the Beveridge plan appeared essential for the organization of health after the end of World War II. It consolidated not only the right to life, but

a different right, more important and complex, represented by the right to health.

With the Beveridge Plan, the State takes on the social task of taking charge of the health of the members of society, with a profoundly different meaning from what had happened up to then. This is a reversal point of view, because the health concern does not longer apply to the State itself and its priorities, but rather individuals. No longer the healthy individual at the service of the State, but the State at the service of the healthy individual. Foucault specifies how a kind of body morality has been defined, which, in the 19th century, had focused on cleaning and hygiene practices as a guarantee of good health. On the contrary, starting from the 20th century, it even goes so far as to support the right to suspend work because of illness. Health and illness thus became a budget item of the State, falling within the government macroeconomic sphere. The Beveridge plan ultimately outlines the idea that the risks, connected with health and the possible interruption of work, no longer concern individuals but the State, which must therefore bear them (148-149). It is precisely on the basis of this approach that the aim of politics takes the name of welfare, because the state of well-being is guaranteed to citizens, who commit themselves to the contractual conditions of a pact, where the sacrifice of work is exchanged for insurance guarantees on health and old age. In this perspective, the management of the economic crisis by the capitalist economy assumes the connotations of a policy of salvation and of almost religious matrix. It is also highlighted by the etymology of the terms "healthy" and "safe", which, within the social reforms of the second post-war period, highlight the overlap between the religious value of salvation and the biological value of health (Esposito, 2015, 67).

Crisis of modern medicine: a game of relational asymmetries

For Foucault, from the time of Constantine, many governments, including European ones up to the 18th century, have distinguished themselves for their theocratic role, characterized by pursuing the salvation of souls as their main objective; this role, however, has finally given way to a somatocracy, in which the main objective of State intervention is the care of the body, physical health, the relationship between health and disease (Foucault, 1997, 205).

What Foucault reflects further on, however, is the crisis that is evident in current medicine, attributable to the distance that would exist between scientific and effectiveness of medicine. In short, the possible negative effects of medicine, including the risk of death, have been represented as a partial consequence of the ignorance of the doctor or of medicine itself, so, the harmfulness was proportional to its non-scientific nature. At the beginning of the 20th century, however, the harmfulness of medicine was linked to its knowledge, to its being a science (206).

The crisis of medicine has been even more marked by the changes in the relationship between doctor and patient, a relationship from the remote past, starting from to the Hippocratic Oath, despite the Hippocratic principles are very current. Beyond the specific principles and contents, however, the Hippocratic Oath highlights the strong relational character, aimed at guaranteeing unconditional respect for the person at the weakest end of the care relationship. Moreover, the Hippocratic care relationship is strongly characterized by an asymmetrical distribution of resources between the person receiving the care and the person providing it, with the former totally subject to the latter. This asymmetry, inherent in the technical contents of the treatment, producing a radical distance between those who administer the treatment and those who receive it, is not, however, an asymmetry that hinders relationality. In fact, it does not lead to prevaricating outcomes, while it should lead to concrete positive solutions, as the result of a real cooperative spirit. (Ruggeri, 2010, 17).

The relational asymmetry that emerges from the doctor-patient relationship is a constant element of any power relationship, as highlighted also in the foucaultian genealogical reconstruction. If this asymmetry, in the context of sovereignty, was clearly structured between the sovereign and his subjects, nevertheless it remains in the biopolitical perspective. Life, even though it is no longer subject to that power of life and death that acts by taking it, is managed because of relationships within a fundamental

asymmetry between those who hold the truth and knowledge, and those who are subject to it. Obviously, even the doctor-patient relationship is not exempt from this mechanism. The medicalization of society itself has been possible by subjects possessing medical knowledge and considered authoritative. They were able to induce certain behaviors and manage certain dynamics into the society. However, the doctor-patient relationship, sometimes defined as paternalistic, provided that the doctor had the power to treat but, at the same time, to choose the therapy considered most suitable for the case in question. The only limit to this power was the fact that the choice had to be made in science and conscience, with the patient's will practically nil (Grassini and Pacifico 2012, 14).

It was only later that the so-called informed consent had on a crucial role. It finds its beginning during medical experimentations, conducted at the beginning of the 20th century. It acquired its greatest fame in the Nuremberg process, from which arose in 1946 the Nuremberg Code, which states the principle that "the voluntary consent of the human being is essential".

It is however important to underline that this Code was developed as a response to the abuses carried out in the extermination camps during the Second World War, highlighting how experimentation was not a strictly therapeutic activity, but that it had the potential to cause physical and psychophysical injuries to individuals.

A different case was in the traditional medical practice, which was not prosecuted in Nuremberg, where the activity of the doctor, aimed at identifying and treating a certain disease following a more or less safe therapy, was justified on the basis of a state of need, real or supposed (13). It is only in the last decades that the principle of informed consent has firmly established in the relationship between doctor and patient, ratifying the end of the paternalistic relationship and the advent of the autonomy of the patient. It was also expressed by the National Committee for Bioethics in its 1992 Opinion "Information and consent to the medical act", which also revealed the close link between the consent that the patient must give and the information that is provided to him.

The philosopher and sociologist Jürgen Habermas, referring to the Freudian analysis of the therapeutic dialogue between doctor and patient, has taken up the peculiarity of the asymmetrical relationship in the distribution of roles, linking it to the potential of communicative action. In this perspective, he defines "therapeutic criticism" a form of argument that can solve cases of self-deception, to which the patient is often victim (Habermas 1997, 78). This is evident in the relationship between the psychotherapist and the patient, where the latter is induced to reflect on himself and on his own situation. Thus, the behavior of a subject appears rational when he can rid himself of his own illusions, that are not the result of an error, but rather of a form of self-deception. For this purpose, it is essential that the patient first acquires an opening towards those who can shed light on what, at least initially, appears to be a form of irrationality. A rational attitude presupposes a willingness to understand and, if there are communication disorders, also a reflection on linguistic rules.

This reflection can be extended to any professional doctor-patient relationship and to all those situations in which the difference between the patient's self-deception and the physician's knowledge is most evident. The concept of communicative action becomes crucial. It is realized every time that an interpersonal relationship is established between at least two subjects capable of action and language (with verbal or extra-verbal forms). The main aim is to reach an understanding that leads to a common agreement on the action plans to pursue. Language, in this process, is fundamental because the level of interpretation of specific situations susceptible to consensus depends on it (157).

The communication process has clearly become indispensable in order to proceed to any medical act, as also underlined by the Code of Medical Deontology in art. 33, which highlights the duty of the doctor to provide the patient with detailed information on the diagnosis and prospects of intervention and treatment.

From informed consent to defensive medicine

The singular and in some ways surprising fact, therefore, emerges from the history of the doctor-patient relationship, especially with reference to the historical period that began in the twentieth century. In such period, medicine acquired a great therapeutic security, attributing to doctors, for the first time in history, the power to effectively treat common diseases. Paradoxically, at this very juncture, the doctor-patient relationship cracked, risking compromising the prestige that doctors had enjoyed until then. Prestige and medical authority, moreover, played a fundamental symbolical role as indicators of the ability and power of a doctor to transmit confidence during his work and in the possibility of recovery to the patient.
Foucault, reflecting on the possibilities that science and medicine offered to populations, especially since the second half of the last century, highlighted how the current medical tools have different effects on population. Although they are not generally considered harmful, tools can nevertheless be incontrollable, forcing the human species to enter a dangerous history, into a field of probability and risks the extent of which cannot be precisely measured. The philosopher from Poitiers emphasized that, although the medical risk, that is the difficult connection to break between positive and negative effects of medicine, is not a novelty, today this risk has entered a new dimension. It is no longer imputable only to the treated subject or to its descendants, but to the entire human species. The possibility of medical and genetic interventions on DNA has placed life in its entirety (no longer the life of an individual or a specific population) in the field of medical impacts, marking the entrance into what Foucault defines as bio-history. In this dimension, human history can modify life and can exert fundamental effects on its process. This can determine one of the main risks of current medicine, the problems of communication from doctors to patients (and "vice-versa"), in addition to the technical anxiety that doctors and biologists feel about their practice and their knowledge.
Apart from the crisis resulting from these dynamics, Foucault believed that there is also the phenomenon of indefinite medicalization, which has led medicine to act outside its traditional field, going beyond the encounter with the patient and the disease. Medicine is increasingly proposed as an act of authority, regardless of the patient's demand (for example, screening policies or the role that doctors and psychiatrists play both in the workplace and in the judiciary). Finally, the subject of medical intervention is no longer exclusively illness, but health in the broadest sense. Thus, all medical interventions, that in general are aimed to improve the health conditions of individuals, respect the principle that the preponderance given to the disease has become a form of general regulation of society.
Lastly, Foucault considered among the characteristics of modern medicine what he defines as the "political economy of medicine". It would not be a novelty, meaning that from the beginning it was precisely the economic problems that determined the medical organization. However, while in the past medicine was assumed as an instrument of conservation and renewal of the workforce for the functioning of modern society, today it directly produces welfare to the extent that health represents a desire for one and a luxury for the others. Health has effectively become an object of consumption and has become a market product. As a result, the body has also entered the market twice, once by wages, when it has sold its workforce, and once by health, where the body comes to be an object of sensations and desires. Incitement to prevention, empowerment and optimization (different screenings, healthy eating regimens, not smoking, improving own individual performance, etc.) cannot, however, be addressed to everyone. This inevitably is the price to be paid, taking the form of exclusive and exclusionary medicalization (Bazzicalupo 2006, 111-113).
In addition, rapid technological and scientific progress has also led to the reduced perception of death as a possible outcome of the disease, but rather as an avoidable complication. This led to paradoxically believe that, in the event of an unfortunate outcome, it is the doctor who handled the clinical case who has committed a mistake and must pay.
Inevitably, there has been a fracture of the doctor-patient relationship, to which many factors

have contributed, including the progressive bureaucratization of medical services, the process of technocratic and political transformation of the health organization, the increasing costs of care. These factors determine the consequent recourse to the assessment of health costs and benefits for patients, and finally the progressive abandonment of the physical approach to the patient, replaced by clinical investigation and by the growing use of technological tools.

Moreover, the increasingly predominant role of the economy and the costs in the current health has become more evident, transforming hospitals into "Health Authorities", in which the health of citizens is a product or rather a commodity, correlated with the payment and/or the refund by the state for hospital services. Consequently, even ethical values are continuously questioned and often subordinated to economic interests.

These changes have led, especially in recent times, to a growing level of complaints related to so-called "malpractice" cases. Claims arising either from patients' greater awareness of health care, or from a considerable increase of economic compensations established in courtrooms, led to a greater readiness by the public to appeal to jurisprudence for medical ligations. The increase in awareness of the right to health when receiving treatment and in expectations of public health facilities has perhaps reached excessive and often unjustified levels. So that medical treatment, which does not produce the desired clinical outcome, is often interpreted by the patient as a mistake, whereas it can simply be scientifically impossible.

Currently, an increasing number of patients use internet to search for diagnoses and treatments (the "e-doctor" phenomenon) going after that to the family doctor or a specialist to confirm their results. By these dangerous behaviors, many potential patients consider internet as a substitute for the family doctor.

The most obvious consequence of this situation is that doctors are increasingly relying on defensive medicine, allowing their diagnostic and therapeutic strategies to be conditioned by "judicial caution" rather than by their scientific beliefs. This has a serious economic impact, resulting from the excessive provision of care and unnecessary recourse to tests and clinical examinations.

In addition to the doctor's fear to be dragged in a court by patients, another reason for defensive medicine increasing phenomenon is the poor organization of complex health structures, in which the reference protocols do not adequately specify roles and responsibilities.

The burden of each judicial case therefore falls on the individual doctor, who is the last link in the chain of organization of the health system. Consequently, doctors daily face with bigger problems than themselves and resort to defensive medicine, which is just an attempt to share the burden of responsibility with others.

Another consequence of the increase in claims on malpractice is the growing cost of insurance premiums, so much so that recent political proposals on medical claims go in the direction of translating in the civil right rather than criminal process. This would lead to an increase in insurance premiums, the cost of which would not be charged to the individual doctors but to the health facility in which they operate. These insurance policies would encourage risk management, adequately supported by the health service, in order to reduce unfair practices and thus keep insurance premiums to a minimum.

Risk management considers all the huge medical complex activities, undertaken to improve the quality of health care and ensure patient safety. Only a proper risk management can lead to substantial changes in clinical practice, making it more suitable to the needs of both patients and healthcare professionals (Toraldo, Vergari and Toraldo 2015).

Already in December 2001, the National Bioethics Committee (NBC), in an opinion on "Purposes, Limits and Risks of Medicine", demonstrated that medical failures are often the most visible aspects of medical practice in the wider sectors of the population. They generate collective reactions expressed and amplified by the media, with legal consequences and demands for individual damages.

The solution to these problems lies first in educating the public of potential patients. The involvement of society requires ethical communication addressed to all citizens, designed to inform them about the nature, possibilities, limits and risks of modern medicine, both in scientific than in practical terms. Adequate communications mean to provide transparent information and news, even when they are unpleasant or

disappointing. Only in a context of effective transparency is possible, according to NBC, to find solutions to legal and bioethical issues, concerning medical responsibility and of strong social relevance.
It would be essential for all the media to consider their aim to correctly inform citizens, also highlighting the differences that, in health terms, inevitably arise in the various geographical, technological and logistical contexts.

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