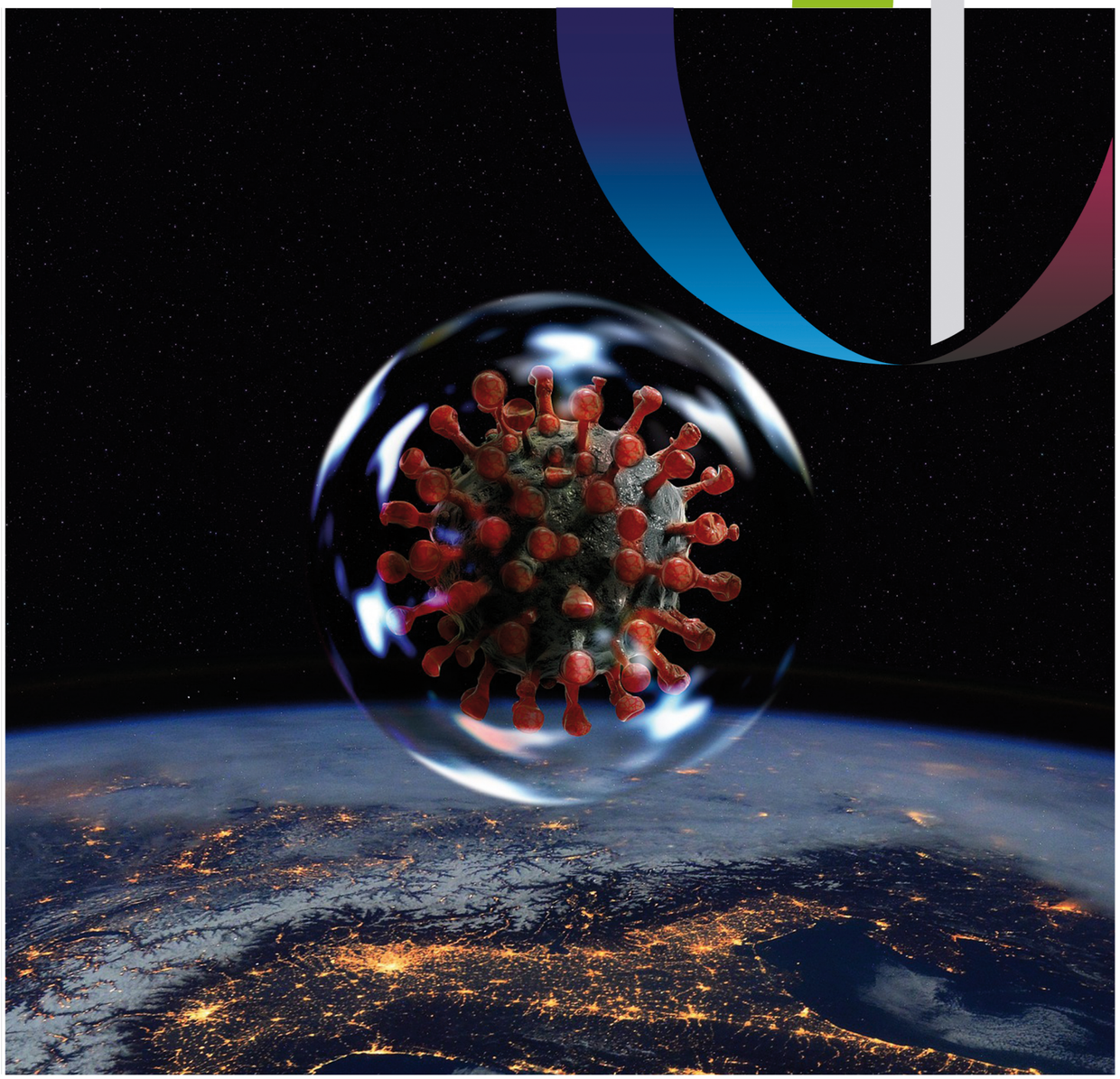
vol. 5 - issue 1 - 2021

Journal of InterDisciplinary Research Applied to Medicine

JDReAM

ISSN 2532-7518
vol. 5 - issue 1
2021
dream.unisalento.it



JDReAM

http://siba-ese.unisalento.it/index.php/jdream

UNIVERSITÀ DEL SALENTO

DREAM
laboratorio diffuso di ricerca interdisciplinare applicata alla medicina

ASL LECCE
SERVIZIO SANITARIO DELLA PUGLIA
DIPARTIMENTO DI SALUTE MENTALE

JDReAM

JOURNAL OF INTERDISCIPLINARY RESEARCH APPLIED TO MEDICINE

VOL. 5 ISSUE 1 (2021)

UNIVERSITÀ DEL SALENTO
2021

JDREAM. JOURNAL OF INTERDISCIPLINARY RESEARCH APPLIED TO MEDICINE
VOL. 5 ISSUE 1 (2021)

eISSN 2532-7518
Journal website: http://siba-ese.unisalento.it/index.php/jdream

JOURNAL OF INTERDISCIPLINARY RESEARCH APPLIED TO MEDICINE

vol. 5 issue 1 - 2021

Index

Life sciences

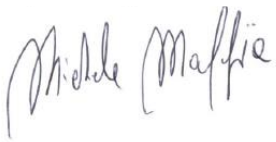
Information and communication, Engineering and Physical sciences

Clinical sciences

Social Sciences and Humanities

The **J**ournal of Inter**D**isciplinary **Re**search **A**pplied to **M**edicine (**J-DReAM**) is one of the editorial aims of DREAM (Interdisciplinary Laboratory of Applied Research in Medicine - University of Salento and the local health authority - ASL Lecce). DREAM is an interdisciplinary laboratory that includes different scientific areas such as biology, biotechnology, biomedicine as well as physics and statistics, computer engineering and bio-materials; it also involves juridical disciplines, political, social sciences and humanities. The purpose of DREAM is to translate the research activities of these scientific areas in clinical settings.

Editor in Chief



Journal of InterDisciplinary Research Applied to Medicine
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5 i. 1, 7-10
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p7
http://siba-ese.unisalento.it, © 2021 Università del Salento

The bidirectional interaction between our gut flora and drugs

Luana Conte[1, 2], Cosimo Caldararo[2], Roberto Lupo[3]

[1]Laboratory of Interdisciplinary Research Applied to Medicine (DReAM),
University of Salento and ASL (Local Health Authority) Lecce, Italy
[2]University of Bari, Bari, Italy
[3]"San Giuseppe da Copertino" Hospital, ASL (Local Health Authority) Lecce, Italy

Corresponding author: Luana Conte
luana.conte@unisalento.it

Abstract

The term "pharmacogenetics" refers to the study of individual genetic variations that give rise to different responses to the intake of a drug. Recently, however, it has begun to think that an important role in this sense can also be played by our microbiota. The interaction between our gut microbial flora and drugs is actually bidirectional: evidence has accumulated that some drugs, in addition to classical antibiotics, have a strong impact on the composition of the microbiota.

Keywords: microbiota, drugs

1. Introduction

The term microbiota refers to the millions of billions of microorganisms (bacteria of thousands of different species, *Archaea*, viruses, and fungi that crowd our organism). These microbes live on the skin, in the mucous membranes of the mouth, in the respiratory tract, but a particularly important role is played by the intestinal component, a diverse and dense microbial community, unparalleled in other bodily habitats. At birth, the digestive tract is almost sterile: the intestinal microbiota is transferred from the mother to the child during childbirth, when it occurs naturally, and this process of colonization continues during the first days of life, with different species depending on whether the infant is artificially or breastfed. It then undergoes a prolonged period of postnatal development, during which it is influenced by contact with external agents such as foods, metals, and toxic substances.

It is estimated that the intestinal bacterial heritage of an adult is composed of over one hundred trillion cells, most of which belong to six bacterial phyla: *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia.* They perform functions essential to our health, such as the breakdown of plant polysaccharides - fibers - indigestible by the host, the detoxification of xenobiotics, substances foreign to the body, the suppression of the growth of harmful microorganisms by competitive exclusion and the development of the host immune system, the biosynthesis of essential vitamins and amino acids. For example, our organism is unable to produce vitamin K, which is essential for the proper synthesis of coagulation factors, and uses, in addition to the diet, that which is produced by some bacteria of our resident intestinal microbial flora.

The healthy pulmonary microbiota contains many commensal bacteria derived from the upper respiratory tract, mainly belonging to the phylum *Bacteroidetes*, especially the genus *Prevotella*, and the phylum *Firmicutes*, especially *Veilonella* and *Streptococcus* (Dickson et al. 2015). Its composition is determined by numerous factors and anatomo-clinical circumstances: a) displacement of bacteria from the upper airways by passive migration, b) gastroesophageal micro-aspiration from the digestive tract c) saprophytic anti-infective activity by the innate and adaptive defenses of the healthy lung, d) by

productive catarrhal cough and altered mucociliary clearance that modulate pathological cellular and bacterial migration (Dickson and Huffnagle 2015).
The healthy lung microbiota acts as a guardian of respiratory health by providing local immunologic defense factors that prevent colonization of opportunistic pathogens by triggering eosinophilic and/or neutrophilic cellular inflammatory processes (Dekaboruah et al. 2020). The dramatic disruption of this respiratory commensal flora (termed pulmonary dysbiosis) in patients with chronic lung disease alters the physiological taxonomic composition resulting in acute inflammation that has been associated with the chronic inflammatory pathogenesis of lung disease.
The balance between our organism and the bacterial flora that inhabits it is therefore valuable, but the composition of the microbiota is highly dynamic and shows substantial inter-individual and intra-individual variations. It is closely related to the age and genetics of the host, but also to environmental factors such as season, smoking, number of hours of sleep, even consumption of carbonated beverages and type of diet (Falony et al. 2016).

2. *How drugs interact with intestinal microbiota*

Evidence has accumulated that some drugs, in addition to classical antibiotics, have a strong impact on the composition of the microbiota.
With few exceptions, drugs do not see their site of action in the intestine but, if they are administered orally, they reach it to be absorbed there and then distributed, thanks to blood-mediated transport, throughout the body. Naturally, it is difficult to determine precisely how much drug will reach the site of action and how long it remains there. Also, for this reason it is difficult to determine what effect it may have on the resident intestinal flora and vice versa.
While it is easy for antibiotics to understand how they can alter the composition of the intestinal flora depending on their spectrum of action, it was more surprising to see evidence of a bactericidal effect on many non-antibiotic drugs. A study published in 2018 analyzed about a thousand non-antibiotic drugs and showed that 24% of them, including mostly agents used in the treatment of mental disorders, antidiabetics, and anticancer agents and inhibited, in vitro, the growth of representative strains of bacterial flora (Maier et al. 2018).
Alterations in the composition of the intestinal flora are linked to mental disorders such as anxiety, schizophrenia, bipolar disorder and depression. Drugs used to treat these illnesses have been found to interact significantly with the microbiota. The first marketed antidepressant, iproniazide, a monoamine oxidase inhibitor, is now used as a drug to treat tuberculosis due to its ability to kill the mycobacterium responsible.
Some widely used antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), have been shown to inhibit the efflux pumps of bacteria and thus prevent their replication. Phenothiazines (and some derivative molecules used as antihistamines), the largest class of antipsychotics, have also been associated with anti-infective properties. In this regard, that of antipsychotic drugs is a particularly interesting chapter: numerous studies have been conducted on their interaction with the bacterial flora, and it has been shown that the administration of atypical antipsychotics as recently introduced psychotropic drugs that are more tolerated than their predecessors, induces dysbiotic alterations to the composition of the intestinal microbiota. Interestingly, there is an inverse relationship, as antibiotic-induced dysbiosis has been linked to mental disorders such as anxiety and depression (Mognetti 2020).
It has been proposed that many of the metabolic side effects associated with the use of atypical antipsychotics, including weight gain, cardiometabolic disorders, and development of the metabolic syndrome, are the result of pharmacological action on the microbiota. For example, long-term exposure to risperidone increases the *Firmicutes/Bacteroidetes* ratio, which is associated with obesity. In addition, a decrease in *Akkermansia muciniphila* has been observed in patients treated with atypical antipsychotics, and this species is known to have beneficial anti-inflammatory effects and to adversely affect fat mass development. An experimental study in mice lacking bacterial flora (germ-free) then revealed that the gut microbiota was responsible for the weight gain observed in response to olanzapine treatment. Similarly, the side effects of these drugs on metabolism were partially re-

versed in female rats when administered concomitantly with an antibiotic (Flowers et al. 2017). Taken together, these results demonstrate that atypical antipsychotics can have a profound impact on host metabolism through their effects on the gut microbiota.

Sometimes the effect of the drug on the composition of the intestinal flora is indirect. Proton pump inhibitors (gastroprotective drugs widely used, for example in the treatment of gastric ulcer or gastroesophageal reflux diseases) modify the pH of the gastrointestinal tract, thus favoring the proliferation of bacterial species that prefer a more basic environment to the detriment of those that prefer an acid environment. Proton pump inhibitor users have been shown to have a larger bacterial flora consisting of more species than non-users (Imhann et al. 2016; Jackson et al. 2016).

The list of non-antibiotic drugs capable of modifying the composition of the intestinal microbiota is long, and includes, among others, hormonal contraceptives, laxatives and antihistamines. However, in many cases we do not know how this happens and if the impact is relevant for the whole flora or only for some bacterial species, also considering other parameters such as contact times and the amount of drug that actually remains in the intestine (Mognetti 2020).

3. Microbiota action on active drugs

Some drugs can modify the composition of the bacterial flora, but numerous studies have shown that contextually, it is capable of modifying many of the active ingredients of the drugs with which it comes into contact, similar to what it is able to do on molecules that we normally introduce with food (Kåhrström, Pariente, and Weiss 2016). Several drugs that can be modified by the gut microbiota have already been identified, including omeprazole, a gastroprotectant, clonazepam, an anxiolytic, the antidiarrheal loperamide, and many others. The list of drugs subject to modification by the intestinal flora is certainly incomplete, since systematic analyses on microbial metabolism are still lacking, also in light of the great inter- and intra-individual variability of the bacterial flora.

A documented example in which the action of a bacterium component of our intestinal microbiota negatively affects the effect of a drug is *Eggerthella lenta*, a bacterium known to possess an enzyme by which it inactivates digoxin, a valuable drug of plant origin used to treat heart failure.

Similarly, other rations that certain bacterial species perform on specific drugs have been identified. Except in rare cases, however, we do not know whether these reactions modify the effect of the drug and in what way. It is possible that bacterial-induced changes in vivo enhance the effect of the drug, in which case they would be beneficial to the user. Or they could be responsible for certain collateral effects, a hypothesis that has been put forward especially for drugs that have repercussions at the gastrointestinal level or that, as in the case of some antipsychotics, cause an increase in body weight. In this sense, is paradigmatic the case of irinotecan, an anticancer drug that our body binds to a molecule called glucuronic acid in order to eliminate it more easily. The complex irinotecan-glucuronic acid, in addition to being less toxic than the original drug, is also more easily removed with the feces. Some bacteria in our intestinal flora are equipped with an enzyme, beta-glucuronidase, which can break down the bond between the drug and the cofactor, thus returning the drug to its original form and toxicity.

Therefore, given the ability of our commensal bacteria to modify the drugs that transit in our intestine, it was thought to intervene on these reactions, to limit them when they are harmful or to exploit them when they are favorable to the host. For example, with reference to the case of irinotecan, researchers have found a way to prevent the activity of beta-glucuronidase with inhibitors that do not modify the composition of the bacterial flora nor give an effect on our organism. This would allow the anticancer drug to be administered while limiting its toxicity. Other interventions have been devised in this sense: for example, it has been demonstrated that modifying through probiotics or antibiotics the composition of the bacterial flora by including some species of gram-positive bacteria, influences the effectiveness of the antineoplastic drug cyclophosphamide (Mognetti 2020).

4. Conclusions

Many studies are focusing on the potential of microbiota-based medicine. Continued advances in the field could lead to targeted approaches to improve drug outcomes by modifying the gut bacterial makeup and to have drug outcomes predicted based on the composition of everyone's flora. However, it will not be easy to incorporate the role of our bacterial flora into toxicology and pharmacology: drug-microbiota-host interactions are inherently complicated and as such require a complex combination of experimental and computational approaches to dissect them. In any case, developing tools, including dietary, or even synthetically engineered probiotics and bacteria through which to manipulate the microbiota for therapeutic purposes would allow us to better respond to a drug or limit its side effects.

5. References

- Dekaboruah, Elakshi, Mangesh Vasant Suryavanshi, Dixita Chettri, and Anil Kumar Verma. (2020). "Human Microbiome: An Academic Update on Human Body Site Specific Surveillance and Its Possible Role." Archives of Microbiology, June, 1–21. https://doi.org/10.1007/s00203-020-01931-x.
- Dickson, Robert P., John R. Erb-Downward, Christine M. Freeman, Lisa McCloskey, James M. Beck, Gary B. Huffnagle, and Jeffrey L. Curtis. (2015). "Spatial Variation in the Healthy Human Lung Microbiome and the Adapted Island Model of Lung Biogeography." Annals of the American Thoracic Society 12 (6): 821–30. https://doi.org/10.1513/AnnalsATS.201501-029OC.
- Dickson, Robert P, and Gary B Huffnagle. (2015). "The Lung Microbiome: New Principles for Respiratory Bacteriology in Health and Disease." Edited by William E. Goldman. PLoS Pathogens 11 (7): e1004923. https://doi.org/10.1371/journal.ppat.1004923.
- Falony, Gwen, Marie Joossens, Sara Vieira-Silva, Jun Wang, Youssef Darzi, Karoline Faust, Alexander Kurilshikov, et al. (2016). "Population-Level Analysis of Gut Microbiome Variation." *Science* 352 (6285): 560-64. https://doi.org/10.1126/science.aad3503.
- Flowers, Stephanie A., Simon J. Evans, Kristen M. Ward, Melvin G. McInnis, and Vicki L. Ellingrod. (2017). "Interaction Between Atypical Antipsychotics and the Gut Microbiome in a Bipolar Disease Cohort." Pharmacotherapy: *The Journal of Human Pharmacology and Drug Therapy* 37 (3): 261-67. https://doi.org/10.1002/phar.1890.
- Imhann, Floris, Marc Jan Bonder, Arnau Vich Vila, Jingyuan Fu, Zlatan Mujagic, Lisa Vork, Ettje F Tigchelaar, et al. (2016). "Proton Pump Inhibitors Affect the Gut Microbiome." *Gut* 65 (5): 740-48. https://doi.org/10.1136/gutjnl-2015-310376.
- Jackson, Matthew A, Julia K Goodrich, Maria-Emanuela Maxan, Daniel E Freedberg, Julian A Abrams, Angela C Poole, Jessica L Sutter, et al. (2016). "Proton Pump Inhibitors Alter the Composition of the Gut Microbiota." *Gut* 65 (5): 749-56. https://doi.org/10.1136/gutjnl-2015-310861.
- Kåhrström, Christina Tobin, Nonia Pariente, and Ursula Weiss. (2016). "Intestinal Microbiota in Health and Disease." *Nature* 535 (7610): 47. https://doi.org/10.1038/535047a.
- Maier, Lisa, Mihaela Pruteanu, Michael Kuhn, Georg Zeller, Anja Telzerow, Exene Erin Anderson, Ana Rita Brochado, et al. (2018). "Extensive Impact of Non-Antibiotic Drugs on Human Gut Bacteria." *Nature* 555 (7698): 623-28. https://doi.org/10.1038/nature25979.
- Mognetti, Barbara. (2020). "Microbiota: A Chemical Laboratory." *Sapere*. Dedalus Editions, 2020. https://doi.org/10.12919/sapere.2020.01.5.

Journal of InterDisciplinary Research Applied to Medicine
JDREAM
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5 i. 1, 11-18
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p11
http://siba-ese.unisalento.it, © 2021 Università del Salento

The Eye-tracking technology in the healthcare settings: an observational, cross sectional, multicenter study

Luana Conte[1,2]; Alessia Lezzi[3]; Roberto Lupo[4] Simone Zacchino[3], Antonino Calabrò[5]; Cosimo Petrelli[4]; Cosimo Caldararo[6]; Antonietta Abate[7]; Alessandra Rizzo[8]; Fiorella Fabrizio[9]; Maicol Carvello[10]; Maria Chiara Carriero[11]

[1]Laboratory of Interdisciplinary Research Applied to Medicine, University of Salento and ASL (Local Health Authority), Lecce, Italy
[2]Laboratory of Biomedical Physics and Environment, Department of Mathematics and Physics, University of Salento, Lecce, Italy
[3]Ant Italia onlus (Associazione Nazionale Tumori) Lecce, Italy
[4]"San Giuseppe da Copertino" Hospital, ASL (Local Health Authority) Lecce, Italy
[5]"Nuovo Ospedale degli Infermi" Hospital ASL (Local Health Authority) Biella, Italy
[6]University of Bari, Italy
[7]"EuroItalia" Centre, Casarano, Lecce, Italy
[8]"Santissima Annunziata Hospital" Asl (Local Health Authority) Taranto, Italy
[9]"Vito Fazzi" Hospital, ASL (Local Health Authority) Lecce, Italy
[10]"Brisighella Community" Hospital, ASL (Local Health Authority) Romagna, Brisighella, Ravenna, Italy
[11]"Santa Chiara" Institute, Rome, Italy

Corresponding author: Luana Conte
luana.conte@unisalento.it

Abstract

Successful communication is considered an essential component of the quality of care and safety of the patient with Amyotrophic Lateral Sclerosis (ALS). Recent technology has provided alternative communication systems, including the Eye-tracking technology, which enables interaction with others in the more advanced stages of the disease. The aim of the study is to investigate patients' difficulties in using the eye tracker, their problem in obtaining the device and the clinical complications resulting from it. A "snowball sampling" method study was conducted from April to September 2020 until sample saturation. The results of the study demonstrate the countless difficulties in obtaining the eye- tracker, with long waiting times, which are not followed by adequate training in its correct use. Among the consequences linked to the use of this device, the most frequent were nystagmus (8.8%), onset of eyelid ptosis (16.2%) and the appearance of increased fatigue. 56.1% of the sample used the eye tracker to surf the Internet whereas 9.1% used it to write e-mails. Overall, the use of the eye tracker led to an improvement in overall quality of life (24%). In Conclusions, the Eye-tracking technology is a valuable device for Alternative Augmentative Communication (AAC) in ALS patients and can be used with good performance, therefore the need for information, training and improvement on this topic is essential

Keywords: Eye-tracking technology, Eye tracker, Amyotrophic Lateral Sclerosis

1. Introduction

The incidence of Amyotrophic Lateral Sclerosis (ALS) is about 1-3 cases per 100,000 inhabitants per year and the relationship between physical impairment and psychological condition is an important issue that involves a direct correlation between severity of depression and physical disability. In recent decades, the progress of biomedical technologies has led to great benefits in the field of healthcare, with the aim of improving the health of individuals and offering alternative solutions to traumas and various diseases. Among these, ALS patients have always used computer media, including computers, writing and digital language systems to communicate with the outside world. (Beukelman and Mirenda, 2014). Studies on the mental evaluation of ALS patients have reported complaints such as depression, anxiety, loss of emotional control and alienation, as well as a high risk of suicide in the early stages of the disease (Cui et al., 2015). In the presence of this debilitating neurodegenerative disease, the

home environment becomes the core of life, where the need to communicate grows and although the progression of language symptoms may vary from person to person, almost all people with ALS have severe communication disorders (Beukelman and Mirenda, 2014). The spread of Alternative Augmentative Communication (AAC) strategies has offered great possibilities to improve the communicative effectiveness of people with ALS, also improving their quality of life. There is a great technological variety of eye-tracker, in computerized systems that are called "Eye-tracking technology". The concept of Eye-tracking refers to a series of technologies that allow the monitoring and recording of the way a person looks at a given scene or image, in which areas they focus their attention, for how long and what order is followed in their visual exploration. Eye-tracking analyses, through a digital camera (eye-tracker), the movement of the eye and in particular the position of the pupil. These devices do not provide new possibilities for curing or improving the disease condition but can improve and increasing the efficiency of responses to some essential needs for autonomy: communicating, working, having fun, and spending free time, controlling the living environment. The eye is usually 'illuminated' by an infrared light emitter that defines the contour of the pupil and creates a light reflection that is captured by the high-resolution camera. These reflections are then processed by special software to calculate the position of the gaze in relation to an object or a position on the computer display. Another feature is the ability to move the head during use. This is only important for those who can still move their head, but not at all for those who cannot. When evaluating instruments, much attention should be paid to the software, as the software represents the 'autonomy functions' that the instrument is able to handle. Eye-tracker-based systems also require a previous calibration process. This new technological availability represents a great opportunity to improve or compensate for those conditions of disadvantage due to disability. In addition to the still excessive cost of these devices, an aspect emerges that is little considered in the studies present in the literature: not all participants are suitable for these studies and the time required for calibration, the difficulty of analyzing and interpreting the results is highlighted as one of the most important barriers (Hassan Montero and Herrero Solana, 2007). The aim of the study is to investigate patients' difficulties in using the eye tracker, their problem in obtaining the device and the clinical complications resulting from it.

2. Methods and design of the study

An observational, cross-sectional, multicentre study was conducted between April and September 2020. The study was carried out through the electronic dissemination of an online questionnaire, as it was considered an effective and inexpensive method to find a significant number of participants among ALS patients. The study was conducted nationwide, through conventional, non-probability sampling. Each patient, contacted through two social networks (private facebook and instagram groups), could join the study through a link to the study. The questionnaire used for data collection consists of two sections: the first section is aimed at collecting socio-demographic information from the patients (age, gender, marital status, educational qualification, profession held before the disease, region and municipality of origin, stage and onset of the disease, heredity, etiopathogenesis) the second section explores everything about the eye-tracker, both the benefits and risks that the use of this device can bring, how to use it and problems that have arisen. All sections of the questionnaire were computerised by using a pre-set form from the Google Drive platform. People affected by ALS, coming from the different Italian regions, who were older than 18 years and who use the eye-tracker for communication and who signed the informed consent were included. Patients who do not use the eye-tracker, who are younger than 18 years old and who have not signed the informed consent were excluded.

3. Ethical considerations

The ethical characteristics of the study were set out in the questionnaire presentation. Participation in the study, being free and voluntary, was considered as an expression of consensus. It was specified that participation was voluntary,

and that the participant could refuse to participate in the protocol whenever he or she wished.

4. Tools

The 68 patients willing to participate in the survey were given an ad hoc online questionnaire (consisting of 21 multiple-choice questions) to obtain more detailed information on the satisfaction and use of this specific aid and the QUEST (Quebec User evaluation of satisfaction with Assistive Technology) (Demers et al., 2000) to have a standardised reference on the degree of satisfaction with the product, the service and the supply, support, and verification service.

5. Social and demographic sample characteristics

The sample consisted of 68 patients affected by ALS with a prevalence of the male gender (76.5%; n=52) and an average age of 57.13 years and DS= 11.729. The prevalence of the sample came from the Puglia Region (29.4%; n=20). The 42.6% (n=29.4) are graduated, and the 29.4% (n=20) are employed. 64.7% (n=44) are married, and the partner appears to mainly take care of them (60.3%; n=41). 82.4% (n 56) receive a pension, which is not enough to cover their care expenses (52.3%; n 34). 63.2% (n=43) move only their eyes (Table 1).

Table. 1 Social-demographic data (n =68)	**N (%)**
Age (average, ds)	57.13 – 11.729
Gender	
Male	52 (76.5)
Female	16 (23.5)
Qualification	
Regional diploma	29 (42.6)
University diploma	14 (20.6)
Middle school	20 (29.4)
Anything/Elementary school	5 (7.4)
Civil status	
Celibate / Maiden	9 (13.2%)
Married	44 (64.7%)
Divorced	6 (8.8%)
Windower	9 (13.2%)
Geographical distribution	
Abruzzo	3 (4.4)
Basilicata	1 (1.5)
Calabria	1 (1.5)
Campania	1 (1.5)
Emilia Romagna	5 (7.4)
Lazio	4 (5.9)
Liguria	3 (4.4)
Lombardia	5 (7.4)
Marche	9 (13.2)
Molise	1 (1.5)
Piemonte	3 (4.4)
Puglia	20 (29.4)
Sardegna	3 (4.4)
Sicilia	4 (5.9)
Toscana	1 (1.5)
Umbria	3 (4.4)
Veneto	1 (1.5)
Professional role	
Artisan	13 (19.1)
Housewife	4 (5.9)
Trader	5 (7.4)
Employee	20 (29.4)
Nurse	1 (1.5)
Engineer	3 (4.4)
Teacher	8 (11.8)
Doctor	5 (7.4)
Worker	9 (13.2)
Caregivers	
Friends	2 (2.9)
Carer	2 (2.9)
Children	18 (26.5)
OSS	1 (1.5)
Partner	41 (60.3)
Brothers/Sisters	3 (4.4)
Healthcare facilities	1 (1.5)
Do you receive a disability pension?	
Yes	56 (82.4)
No	12 (17.6)
Is the pension sufficient to cover the costs of providing care needs?	
Fairly	14 (21.5)
Slightly sufficient	34 (52.3)
Not sufficient	17 (26.2)
Missing	3 (4.4)
Depending on the stage of the disease, which part of the body can still move?	
Regional course	11 (16.2)
University Diploma	2 (2.9)
Master's Degree	7 (10.3)
Bachelor's degree	43 (63.2)
Master 1st Level	5 (7.4)

6. Communication system and the use of the eye tracker

92.6% (n=63) were immediately in favor of using the eye-tracker. 51.5% (n=35) used other systems for accessing the PC prior to the eye-tracker, of which 37.8% (n=14) were reported

to have used head-controlled mouse emulators, while 36.8% (n=25) used alphabetical communication tables as other systems for AAC. 42.6% (n=29) became aware of the eye-tracker through their medical specialist (neurologist, physiatrist), whose requirements for the use of the eye-tracker were assessed (30.9%; n=21). The test for the use of the eye-tracker was carried out both at home (45.6%; n=31) and in hospital (47.1%; n=32), and only one type of eye-tracker was used (73.5%; n=50). The prescription was made by the NHS specialist doctor (Neurologist, Otolaryngologist, Physiatrist) (55.9%; n=38), with long timescales (57.4%; n=37). 32.4% (n=22) had been using the eye-tracker for 4-12 months. Only 5.9% (n=4) had such difficulties during training that they were unable to use it and 39.7% (n=27) were able to use it "always" or "quite a bit" after training (39.7%; n=27) without difficulty. The technical problems associated with the device that make it difficult to use are the need for continuous recalibration (33.8%; n=23). 69.1% (n=47) also use the eye-tracker for other purposes in addition to communication, for surfing the Internet (56.1%, n=37). Over the course of the day, 36.8% (n=25) spent between 2 and 5 hours using the eye gaze eye-tracker, of which 36.8% (n=25) were in bed. Using the eye-tracker caused 41.2% (n=28) eye fatigue, but 30.9% (n=21) had no health problems that made its use necessary. Only 8.8% (n=6) were not at all satisfied; 41.2% (n=28) felt that the device had brought "enough" improvement in the overall quality of their lives. The 33.3% (n=19) would ask for a greater health comfort (Table 2).

Table. 2 The Eye-tracking technology use	**N (%)**
Were you immediately in favor of using the eye tracker?	
Yes.	63 (92.6)
No	5 (7.4)
Before using the eye tracker, did you use other systems to access the PC?	
Yes	35 (51.5)
No	33 (48.5)
If yes, please indicate which ones:	
Membrane mouse emulators	7 (18.9)
Scanning mouse emulators	4 (10.8)
Head-controlled mouse emulators	14 (37.8)
Computer voice command software	4 (10.8)
On-screen keyboards	4 (10.8)
Reduced keyboards with mouse emulation	4 (10.8)
No other systems used	37 (56.1)
Have you used other systems for AAC (Alternative Augmentative Communication)?	
Portable alphabetic communicators	8 (11.8)
Alphabetic communication tables	25 (36.8)
VOCAS	17 (25.0)
No system	18 (26.5)
How did you find out about the eye tracker?	
Other patients	4 (5.9)
Acquaintances	5 (7.4)
Media	13 (19.1)
Attending physician	1 (1.5)
Specialist doctor (neurologist, physiatrist)	29 (42.6)
Internet sites or magazines dedicated to disabled people	16 (23.5)
Who carried out the assessment of your requirements for the use of the eye tracker?	
Specialist outpatient clinic of your local health authority	15 (22.1)
Specialist outpatient clinic of another health authority	14 (20.6)
Specialist doctor	21 (30.9)
Technician of the supplier company	18 (26.5)
In which setting was the eye tracker test carried out?	
At home	31 (45.6)
In an outpatient clinic	5 (7.4)
Hospital	32 (47.1)
Did you try only one type of eye-tracker or more than one?	
Do not remember	3 (4.4)
More than one	15 (22.1)
Only one type	50 (73.5)
Please indicate other models:	
Dialog and Eyetech	1 (6.7)
Dylog	2 (13.3)
Eye Gaze	3 (20.0)
Medicair	1 (6.7)
MyTobii	1 (6.7)
PC	1 (6.7)
The Grid 3	2 (13.3)
Tobii Dynavox	3 (20)
Vivisol	1 (6.7)
Missing	53 (75.0)
Who prescribed it?	
Territorial district director	8 (11.8)
Dedicated outpatient specialist doctor	21 (30.9)
NHS specialist (Neurologist, Otolaryngologist, Physiatrist)	38 (55.9)
None (on loan)	1 (1.5)
What difficulties did you encounter in obtaining the device?	
Long delays	39 (57.4)
District refusal	4 (5.9)
Delivery of another type of device	10 (14.7)
Other (please specify...)	15 (22.1)
How long have you been using the	

eye tracker?	
1-3 months	11 (16.2)
4-12 months	22 (32.4)
12-36 months	21 (30.9)
>36 months	14 (20.6)
Did you find any difficulties in training to use it?	
Many difficulties	13 (19.1)
So many difficulties that you could not use it	4 (5.9)
No, none	24 (35.3)
Some difficulty	27 (39.7)
How long did the training take?	
One day	17 (25.0)
A few days	15 (22.1)
One month	13 (19.1)
Several months	23 (33.8)
Can you use it easily?	
Fairly	27 (39.7)
I still have some difficulties	12 (17.6)
Yes, all the time	27 (39.7)
I find it too difficult to use	2 (2.9)
Have you encountered technical problems with the device that make it difficult to use?	
Machine malfunction	17 (25.0)
Need for continuous recalibration	23 (33.8)
Positioning and installation	13 (19.1)
No problems	15 (22.1)
Are you satisfied with the way and speed with which you can communicate your care needs using the eye tracker?	
Very satisfied	3 (4.4)
Fairly satisfied	40 (58.8)
Satisfied	19 (27.9)
Not at all satisfied	6 (8.8)
Can you use the eye pointer for purposes other than communication?	
No	15 (22.1)
No, but I would like to	6 (8.8)
Yes	47 (69.1)
If the answer is "yes" or "no, but I would like to" please specify:	
Environmental control	4 (6.1)
Managing files such as photos, videos, films, books, documents	14 (21.2)
Work, study, research	4 (6.1)
Send text messages	1 (1.5)
Surfing the internet	37 (56.1)
Writing e-mail	6 (9.1)
Missing	2 (2.9)
How many hours per day do you spend using the eye-tracker?	
Less than 2 hours	12 (17.6)
Between 2 and 5 hours	25 (36.8)
Between 5 and 10 hours	20 (29.4)
More than 10 hours	11 (16.2)
What position do you assume when using the eye reacker?	
Sitting in a wheelchair	23 (33.8)
In bed	25 (36.8)
In both	20 (29.4)
Has using the eye tracker caused problems for your eyes?	28 (41.2)
Fatigue	6 (8.8)
Headache	8 (11.8)
Conjunctivitis	14 (20.6)
Dehydration	12 (17.6)
None	
From the beginning of the use of the eye pointer to the present day, what health problems make its use difficult?	
Redness and tearing	1 (1.5)
Burning in the eyes	2 (2.9)
Occurrence of increased fatigue	7 (10.3)
Difficulty constructing words and reduced ocular mobility, which makes it difficult to optimize dwell time on the letters	1 (1.5)
Onset of nystagmus (difficulty in keeping one's gaze fixed)	19 (27.9)
Onset of palpebral ptosis (lower upper eyelid)	6 (8.8)
Reduction in ocular motility	11 (16.2)
No problems	21 (30.9)
Do you think that the device has led to an improvement in the overall quality of your life?	
Yes, very much	24 (35.3)
Somewhat	28 (41.2)
A little	15 (22.1)
No, not at all	1 (1.5)
Are there any further requests, in general?	
Yes	37 (78.7)
No	10 (21.3)
Missing	1 (1.8)
If yes, please indicate type of request	
More assistance to relieve my wife	1 (1.8)
More home cares	1 (1.8)
More health comforts	19 (33.3)
More care prepared for ALS	12 (21.1)
More heart in the hands of caregivers	10 (17.5)
More care supports	13 (22.8)
More care by qualified staff	1 (1.8)
Missing	11 (16.2)

7. Discussion

The aim of the study was to investigate the patients' difficulties in using the eye-tracker, the problems in obtaining the device and the resulting clinical complications. The study shows a clear predominance of men over women. This is in accordance with literature, where the disease affects both sexes, with a slight prevalence of men (Trojsi et al., 2012) whereas there is an

equal frequency between the two sexes in family forms (Mora and Chiò, 2010). The mean age of our study was 57.13 years, which is in line with other Italian studies (Borasio et al., 2005; Trojsi et al., 2012) in contrast to another Italian study in which the average age was over 65 years old (Garzillo et al., 2015). Rarely are cases of juvenile ALS with onset under 20 years of age and in 5% more cases can be observed in the same family nucleus, often with autosomal dominant inheritance (familial ALS). The various risk factors investigated in the various studies include cigarette smoking, mechanical trauma, intense physical activity and sport, exposure to toxic substances (pesticides, herbicides, some insecticides), heavy metals (mercury, lead, arsenic) and electromagnetic fields. Studies are therefore focusing on finding genetic factors that alter individual susceptibility to external stimuli and thus modify the risk of developing the disease. ("A. Gemelli" et al., 2016). ALS is an event that affects not only the patient, but also the whole family that is forced to face relational, social, and organisational discomfort daily. As highlighted by the study, it is often the partner who takes on the role of caregiver (58.9%; n=40). Numerous studies have investigated the importance of the caregiving activity carried out by caregivers and the effects on the quality of life and psychophysical well-being generated by being constantly in contact with the invalidating disease of a family member or friend. One study in the literature investigated the caregiving needs of the caregiver of a patient with a severe disability, especially in the early stages of the caregiving role, and found that the caregiver felt a greater need for emotional and social support, communication, and information. (Moroni et al., 2008). Patients suffering from ALS need high-intensity health and social care, as well as environmental retrofitting, to first adapt their home to a person in a wheelchair or bedridden, and then to provide artificial ventilation. Having adequate economic resources and space makes a difference in terms of opportunities for care and quality of life. These costs can be transformed into unequal opportunities for access to cure and to-care support, given that the number of hours of home care provided by the public system is often largely insufficient. (Bosco and Cappellato, 2016). In fact, in the study it emerged that 82.4% (n=56), expressed that they receive an invalidity pension, compared with 17.6% (n=12) who expressed that they do not receive it at all, and of that population who stated that they receive an invalidity pension, 47.1% (n=32) and 38.2% (n=26) complained that it is insufficient or even not sufficient to cover the expenses to guarantee the best possible care needs. In the second section, everything concerning the eye-tracker was evaluated: benefits and risks that the use of such a device may provide, ways of using it and problems that have arisen. The possibility of using eye-tracking PC interface systems has offered a new possibility in enabling communication in subjects with severe motor disabilities, such as those who are unable to communicate verbally in oral and written form. It is therefore essential to make use of the resources that the subject possesses, including eye movement, one of the few parts of the body that subjects can still control (63.2%; n=43), around which the eye-tracker technology was created, and which immediately received favourable opinions among the patients in this study (92.6%; n=63). The evaluation of the requirements to use the eye-tracker was carried out by the specialist doctor of the local health authority (30.9%; n=21) as well as the prescription, data in line with another Italian study (Gasperini M.; 2010). The place where the test was carried out appears to be divided between hospital and home, unlike another Italian study in which home was the unanimous answer. Regarding the model of eye tracking device, only 22.1% (n=15) confirmed having used more than one. Each tracker has its own characteristics that should be evaluated based on factors such as the subject who is going to use it, the presence of a monocular/biocular tracker or one that allows access to other multimedia systems. There are no eye-tracker that are better than others, but simply subjects with different needs. According to our data the most used eye-tracker is the Tobii Dynavox (4.4%; n=3) and the Eye Gaze (4.4%; n=3), while 77.9% (n=53) of the sample did not answer. This could lead to the hypothesis that not all the subjects were made aware of the model with which the test was carried out, nor were they aware of other models with different characteristics, which would perhaps be essential for that subject rather than another. Moreover, not

all the devices are equipped with automatic re-calibration; in fact, the need for continuous re-calibration appears among the technical problems related to the device that make its use difficult (33.8%; n=23). Despite the difficulties during training, 79.4% (n=54) once they have learnt how to use the ring eye-tracker, they are able to use it with ease, even for other purposes besides communication, such as surfing the Internet (56.1%; n=37), but despite this 36.8% (n=25) state that they still use alternative systems for alternative augmentative communication, such as alphabetical communication tables. However, some typical eye movement problems such as nystagmus and strabismus, mydriasis or miosis and eyelid ptosis can interfere with eye tracking. For this reason, some eye-tracker have correction filters to compensate for these problems. To use this type of instrument, there must also be adequate ocular vision, i.e., the absence of objective eye problems such as double vision, cataracts, etc., the ability to maintain the position in front of the monitor, cognitive skills appropriate to the task, such as being able to read and memorise the procedures necessary to use the various functions, and the motivation and interest of the user (Gasperini et al. 2011). The survey also assessed the clinical complications encountered, considering the months of use, which vary from 1 month to >36 months. The complication found by most of the sample (41.2%; n=28) was fatigue, which can be related to the hours of daily use of the device, 2-5 hours for 36.8% (25), and 5-10 for (29.4%; n=20) data in line with another Italian study (Gasperini et al. 2011). Complications according to 21% are not such as to make their use difficult. 51.5% of the population were quite satisfied with the way and speed with which they were able to communicate their care needs and complex thoughts using the eye-tracker and 76.5% stated that the new eye-tracking technology had led to an improvement in the overall quality of their lives.

8. Limits of the study

The limitations of the study may be represented by possible selection bias and by a small sample not representative of the entire Italian population affected by ALS.

9. Conclusions and perspective

The aim of the study was to investigate the patients' difficulties in using the eye pointer, the problems in obtaining the device and the resulting clinical problems. The results obtained, confirming previous studies in the literature, show how long waiting times are for obtaining the device and some difficulties in training its use. In addition, clinical complications arising from the use of the device, such as nystagmus, reduced ocular motility, eyelid ptosis, fatigue, redness, lacrimation, ocular dehydration, conjunctivitis, and headaches. But the principal aspect that emerges is precisely how the ocular pointer has proved, in the end, capable of improving the global quality of life of every ALS patient, making it possible to communicate all care needs and complex thoughts with the outside world. In front of these results, it is necessary to reach an important conclusion: the eye-tracking computer system is a valuable device for CAA in ALS patients and can be used with good performance, so the need for information, training and improvement on this issue is essential. This will be an important step towards eliminating the difficulties encountered and therefore less stress and isolation for each individual patient. This study highlighted very interesting aspects about a patient population with an extreme need to communicate. There are very few studies in the literature on alternative communication, so it would be good to explore this aspect further in multi-center, prospective studies.

10. References

- Beukelman, D.R., Mirenda, P., (2014). "Manuale di comunicazione aumentativa e alternativa: interventi per bambini e adulti con complessi bisogni educativi". *Edizioni Centro Studi Erickson.*
- Borasio, G., Voltz, R., Miller, R., (2005). "Le cure palliative nella Sclerosi Laterale Amiotrofica". Riv Ital Cure Palliat 2, 26-39.
- Bosco, N., Cappellato, V., (2013). Malati di SLA in Italia e meccanismi di diseguaglianza". 91-100 https://doi.org/10.13128/cambio-19288

- Cui, F., Zhu, W., Zhou, Z., Ren, Y., Li, Y., Li, M., Huo, Y., Huang, X., (2015). "Frequency and risk factor analysis of cognitive and anxiety-depressive disorders in patients with amyotrophic lateral sclerosis/motor neuron disease". *Neuropsychiatr Dis Treat* 11, 2847–2854. https://doi.org/10.2147/NDT.S90520
- Demers, L., Weiss-Lambrou, R., Ska, B., (2000). "Item Analysis of the Quebec User Evaluation of Satisfaction with Assistive Technology (QUEST)". *Assistive technology : the official journal of RESNA* 12, 96–105. https://doi.org/10.1080/10400435.2000.10132015
- Policlinico A. Gemelli, Ospedale San Filippo Neri, policlinico Umberto I, A.O. San Camillo Forlanini, (2016). "Sclerosi Laterale Amiotrofica, percorso diagnostico terapeutico assistenziale".
- Garzillo, E., Miraglia, N., Pedata, P., Feola, D., Sannolo, N., Lamberti, M., (2015). "Exposure to metals and other occupational/environmental risk agents related to amyotrophic lateral sclerosis: A review of literature". *Giornale italiano di medicina del lavoro ed ergonomia XXXVII*, 8–19.
- Gasperini, M, Menini E., Robol E., Crimi, G; (2011). "Puntatori oculari; valutazione del percorso dalla prescrizione all'outcome in un campione di pazienti neurologici del Veneto: att del 39°" SIMFER Congress.
- Hassan Montero, Y., Herrero Solana, V., (2007). "Eye-Tracking en Interacción Persona Ordenador". *Eye-Tracking en Interacción Persona Ordenador 3*, 10–12.
- Moroni, L., Hamann, D., Paoluzzi, L., Pieper, J., de Wijn, J.R., van Blitterswijk, C.A., (2008). "Regenerating articular tissue by converging technologies". *PLoS One* 3, e3032. https://doi.org/10.1371/journal.pone.0003032
- Trojsi, F., Sagnelli, A., Vanacore, N., Piccirillo, G., Daniele, L., Izzo, F., Laiola, A., Di Costanzo, A., Savettieri, G., Monsurrò, M.R., Tedeschi, G., (2012). "Clinical features and lifestyle of patients with amyotrophic lateral sclerosis in Campania: brief overview of an Italian database". *Ann Ist Super Sanita* 48, 287–291. https://doi.org/10.4415/ANN_12_03_09

Journal of InterDisciplinary Research Applied to Medicine
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5 i. 1, 19-26
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p19
http://siba-ese.unisalento.it, © 2021 Università del Salento

Polygraphic findings in simplified Barbed Reposition Pharyngoplasty (BRP) as a treatment for OSA patients

Caterina Arigliani[1,], Michele Arigliani[2,*], Enrico Ciavolino[3,4], Luana Conte[5,6], Domenico Maurizio Toraldo[7], Silvia Passariello[8], Serena Arima[3], Paola Angelelli[9], Luigi Macchitella[9], Antonio Palumbo[2], Michele De Benedetto[2]*

**These authors contributed equally to this work.*

[1]Plastic and Aesthetic surgery, University of Milan, Milan, Italy
[2]ENT Unit, "V.Fazzi" Hospital, ASL (Local Health Authority) Lecce, Italy
[3]Department of History, Society and Human Studies, University of Salento, Lecce, Italy
[4]Wyższa Szkoła Bankowa w Gdańsku, aleja Grunwaldzka 238A, 80-266 Gdańsk, Poland
[5]Laboratory of Interdisciplinary Research Applied to Medicine (DReAM),
University of Salento and ASL (Local Health Authority) at the "V Fazzi" Hospital, Lecce, Italy
[6]Laboratory of Advanced Data Analysis for Medicine (ADAM), Department of Mathematics and Physics "E. De Giorgi",
University of Salento, Lecce, Italy
[7]Department of Rehabilitation, Cardiorespiratory Rehabilitation Unit, "V.Fazzi" Hospital, ASL (Local Health Authority) Lecce, Italy;
[8]Department of Pharmaceutical Sciences, University of Pavia, Pavia, Italy
[9]Department of History, Society and Human Studies –Lab of Applied Psychology and Intervention - University of Salento, Lecce, Italy

Corresponding author: Caterina Arigliani
cate.arigliani10@gmail.com

Abstract

This study aims to compare polygraphic data in patients with OSA treated with Barbed Reposition Pharyngoplasty (BRP) performed with a simplified technique compared to the standard method. Variations of technique were performed and tested with the purpose of promoting tolerability and diffusion of this simplified technique. To evaluate the efficacy of the simplified BRP method, a sample of 99 patients was divided into two groups: Group A was treated with BRP (BRP group) and Group B was treated with simplified BRP (sBRP group). The results obtained on the two groups were compared with the two sample Bootstrap t-tests method, showing a substantial overlap in polygraphic results recorded 6 months after surgery.

Keywords: Barbed reposition pharyngoplasty, Obstructive Sleep Apnea, polygraphy, Home Sleep Apnea Testing device, sleep surgery

1. Introduction

Obstructive sleep apnea (OSA) is a condition characterized by partial or complete repeated obstructions due to the collapse of the upper airway during sleep (Strollo and Rogers 1996). These cause intermittent hypoxia which leads to systemic damages and high risk of morbidity and mortality (Yaggi et al. 2005).
Surgical treatment for OSA uses different and specific options for each level of upper airway obstruction (Georgalas et al. 2010). One of the surgical options for patients with obstruction at the retro-palatal level is Barbed Reposition Pharyngoplasty (BRP), proven to be an effective and safe technique (Vicini et al. 2015; Montevecchi et al. 2018), that is showing wide success (Dachuri et al. 2019). This technique uses suture materials (Knotless Tissue-Closure Device) (Alessandri et al. 2010) never before used in the surgical therapy for OSA and the surgical rationale introduced by Mantovani (Mantovani et al. 2012; Rinaldi, Mantovani, and Pignataro 2017). The premise of the present study is the experience gained from dissection courses and didactic simulations of cadaveric surgical techniques at the palatal level for OSA, which for us constitute the best opportunity to test alternative surgical solutions. In this study we aimed to evaluate the polygraphic results of some simplifications of technique that were supposed to have low-medium impact on the results but that

made the procedure easier to perform for less experienced surgeons and wanting with this to further promote the diffusion of this type of surgery.

2. Material and Methods

99 Patients were recruited into the sleep apnea surgery protocol after being subjected to Drug-induced sleep endoscopy (DISE) procedure from April 2015 to December 2019. Patients' characteristics have been described in (Arigliani et al. 2021).
All patients had never previously undergone other surgery for OSA but had undergone verification of surgical indications by DISE (Kotecha and De Vito 2018) through the 5VsEs instrumentation (Arigliani et al. 2020). A retrolingual obstruction was excluded through DISE procedure. All patients in this study performed tonsillectomy and palatal surgery only.
Of the 99 patients enrolled, 50 had been treated with standard BRP method (BRP group), 49 had been treated with simplified BRP method (sBRP group).
Unattended set polygraphy, DISE and all diagnostic procedures pre- and post-surgery were performed at "Vito Fazzi" Hospital in Lecce (Italy) by the same operator and with the same instrumentation. All surgical procedures were also performed by the same operator. Post-surgical polygraphic evaluation was performed after at least six months for both groups of patients in the study. For this study, we collected the anthropometric data as well as the pre- and post-surgery polygraphic data (Apnoea Hypopnea Index (AHI), (hour/sleep), Oxygen Desaturation Index (ODI), (hour/sleep), Lowest O2 saturation, (%). We based on AASM guidelines 2007 to make the results of our study comparable with previous literature (Berry et al. 2012).
We used the same protocol for post-operative pain management on all patients: the assessment also included an estimate of post-surgical pain on day 3 using a visual Analogue pain scale (Pain VAS scale) (Chiarotto et al. 2019; Williamson and Hoggart 2005).
For the polygraphic evaluation and post-surgery in both study groups refers to (Arigliani et al. 2021).
Research approval was obtained through the ethics committee of the Local Health Authority (ASL LE) at the Vito Fazzi Hospital (verbal no. 43, 3 March 2020), and informed written consent was obtained from all participants. All international ethical standards were respected throughout the study.

2.1. Surgical technique

The surgical procedure for what concerns the standard group was performed respecting as rigorously as possible the surgical technique published by Vicini et al (Vicini et al. 2015). Regarding the sBRP group, the surgical procedure presents a simplification of technique theoretically of medium-low impact on the obtainable results compared with those of standard BRP.
In the sRBP group, we used a Unidirectional Barbed, dual angle, absorbable Knotless wound closure device in Copolymer of glycolic acid and trimethylene carbonate, 30 cm, single needle, needle 37 mm, 1/2 circle, size 0, taper) (Covidien V-Loc 180™) (Alessandri et al. 2010) (Covidien IIc, Mansfield, MA, USA) instead of a Bidirectional (whith transition zone in the middle) Tissue-Closure Device, double needle, in polydioxanone absorbable monofilament, size 0, recommended for suturing both pharyngeal lateral walls in the original description of the BRP procedure.
The sBRP procedure, on the other hand, stops at step 7 and does not continue to step 8 as in the case of the BRP procedure (Figure 1).
In all cases, an additional suture loop was performed to reinforce step 7.
A monopolar or dipolar diathermy was used. Using a second suture thread Barbed dual-angle, Unidirectional, single needle, absorbable, 30 cm, (Covidien V-Loc 180™) (Alessandri et al. 2010) we performed in the same way the sBRP procedure from the opposite side, taking care to balance and managing pulling force between the two sides.

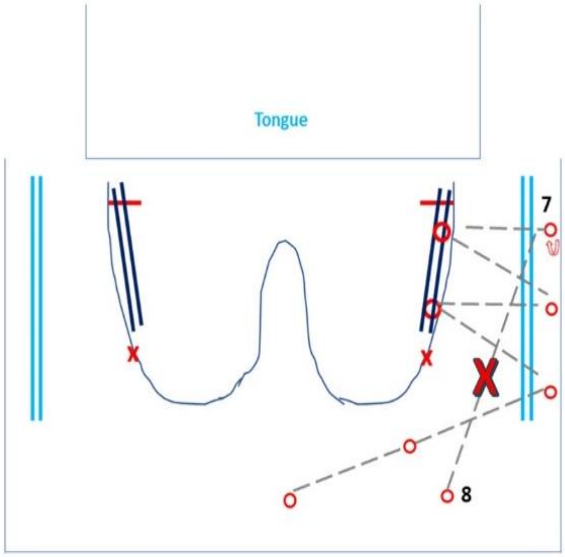


Figure 1. The simplified sBRP procedure with abolition of step 8 compared to the standard BRP procedure. Pterygomandibular Raphe (light blue). Palatopharyngeus Muscle (dark blue).

3. Data Analysis

The sample analyzed is based on 99 patients divided into two groups. In the following subsections the groups are called Group BRP and Group sBRP and a descriptive study is reported for both and then a two sample bootstrap t-test (Brandley and Tibshirani 1993) (Brandley and Tibshirani 1993) is performed with aim to evaluate if there is:

1. A statistical difference between the means for Pre and Post Surgery, in Groups BRP and sBRP, for the following Variables (AHI, BMI, ESS, Lowest SpO_2, ODI).
2. A statistical difference between the means Groups BRP and sBRP, in pre- and post-surgery, for the following Variables (AHI, BMI, ESS, Lowest SpO_2, ODI).

3.1 Descriptive Analysis Group BRP

Table 1 the sample collected (n=50) for the Group A BRP for the five variables measured on the patients for the pre-surgery and post-surgery conditions. For each variable and condition the min, max, mean and standard deviations are reported.

Table 1. Descriptive Indexes group BRP

	Pre				
Variable	*n*	*min*	*max*	*mean*	*sd*
AHI	50	2	70	26,32	15,428
BMI	50	20	34	28,38	2,664
ESS	50	6	16	11,14	2,02
LowSpO2	50	50	94	78,4	10,188
ODI	50	2	75	27,2	15,041
	Post				
Variable	*n*	*min*	*max*	*mean*	*sd*
AHI	50	2	21	7,22	3,507
BMI	50	16	39	27,96	3,865
ESS	50	7	16	9,46	1,681
LowSpO2	50	67	95	84,58	6,263
ODI	50	1	37	12,52	8,922

Body mass index (BMI), Epworth Sleepiness Scale (ESS), Overnight polygraphic values are defined according to the American Academy of Sleep Medicine (AASM) (Berry et al. 2012) the Apnea-Hypopnea Index (AHI) (hour/sleep), oxygen desaturation index (ODI) (hour/sleep), and Lowest saturation O_2 (%).

Figure 2 shows the box-plots for both the phases of the surgery (pre and post) for all the variables. The box-plot helps to read both the central tendency of the variable analyzed (the bold line in the middle of the box is the median) and the variability (based on the size of the box). It is possible to see that all the measures decrease, with the exception of Lowest SpO2, with a reduction of the variability, showing an improvement of stability of the measures.

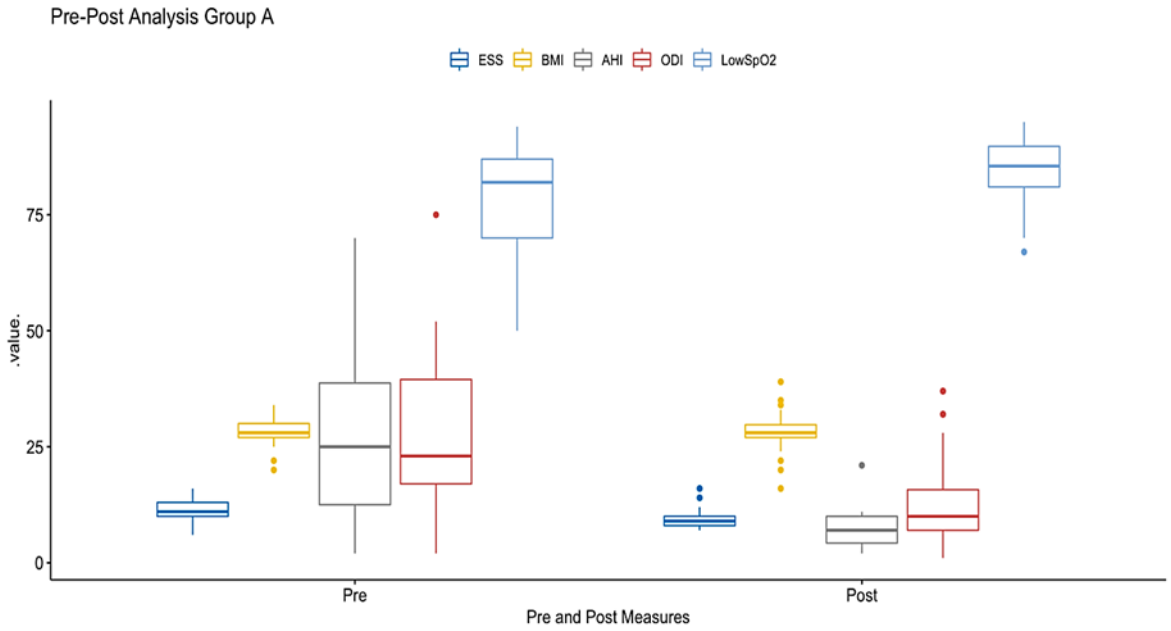


Figure 2. Box-Plot Group BRP

3.2 Descriptive Analysis Group sBRP

In Table 2 are reported the patients' measures of the Group B with a sample size n = 49, for both pre-surgery and post-surgery conditions. For each variable and condition the min, max, mean and standard deviations are reported.

Table 2. Descriptive Indexes group sBRP

	Pre				
Variable	*n*	*min*	*max*	*mean*	*sd*
AHI	49	2	57	23,592	12,297
BMI	49	20	32	27,939	2,794
ESS	49	8	16	10,939	1,983
LowSpO2	49	50	94	80,061	9,675
ODI	49	2	52	21,796	11,13
	Post				
Variable	*n*	*min*	*max*	*mean*	*sd*
AHI	49	1	16	7,408	3,397
BMI	49	20	39	27,714	3,512
ESS	49	7	16	9,388	1,643
LowSpO2	49	68	95	85,694	5,713
ODI	49	2	34	11,592	7,328

Body mass index (BMI), Epworth Sleepiness Scale (ESS), Overnight polygraphic values are defined according to the American Academy of Sleep Medicine (AASM) (Berry et al. 2012) the Apnea-Hypopnea Index (AHI) (hour/sleep), oxygen desaturation index (ODI) (hour/sleep), and Lowest saturation O2 (%).

The visual analysis of the central tendency and the variability is shown by the box-plot reported in Figure 3 for both the phases of the surgery. The group B shows, as for the group A, a reduction of the median, with the exception of Lowest SpO2, and also of the variability for all the variables considered.

3.3 Test the differences in Pre and Post surgery phases

Tables 3 and 4 reports the results of a Bootstrap paired t-test for both Group BRP and Group sBRP. Tables reports in the rows the variables and in the columns: difference between the means, t-test, confidence intervals and p-value. For all the variables, the differences between the means of pre-surgery and post-surgery are statistically significant, with the only exception of the BMI. The results show that the operation reduces all the parameters analyzed, only Lowest O2 increases, with an improvement of the general health conditions.

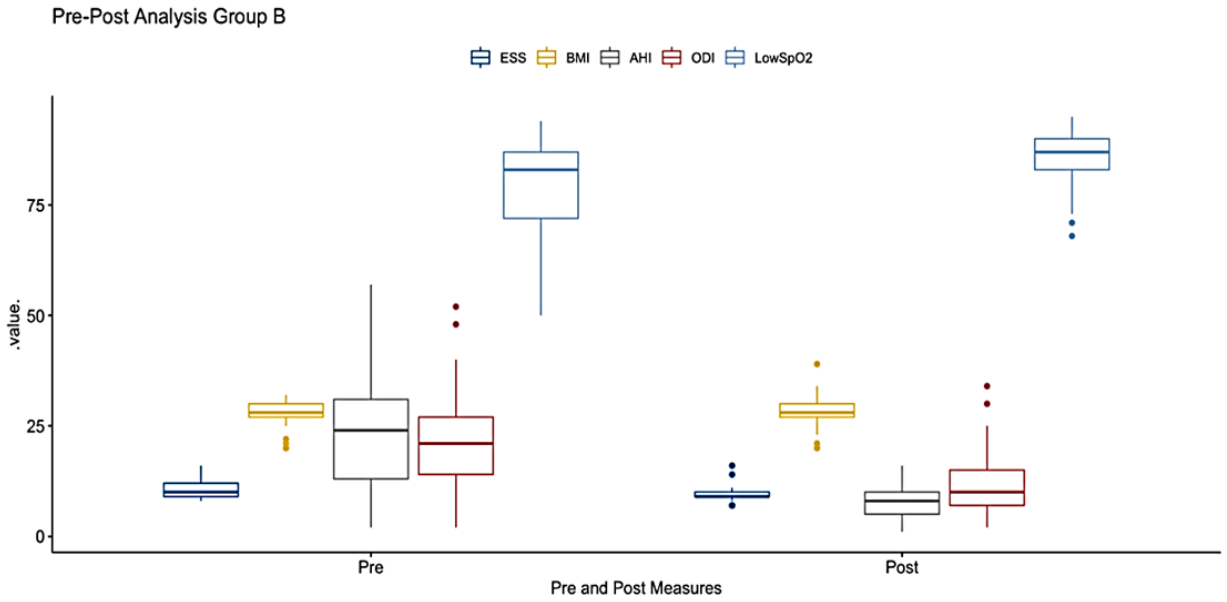


Figure 3. Box-Plot Group BRP

Table 3. Bootstrap paired t-tests. Group BRP

Variable	*mean of the differences*	*t*	*2.5%*	*97.5%*	*p-value*
ESS	-1,68	-7,382	-2,14	-1,26	0
BMI	-0,42	-1,130	-1,16	0,28	0,278
AHI	-19,1	-9,334	-23,16	-15,179	0
ODI	-14,68	-8,139	-18,3	-11,22	0
LowSpO2	6,18	5,619	4,18	8,38	0

Table 4. Bootstrap paired t-tests. Group sBRP

Variable	*mean of the differences*	*t*	*2.5%*	*97.5%*	*p-value*
ESS	-1,551	-5,836	-2,082	-1,041	0
BMI	-0,224	-0,688	-0,816	0,449	0,564
AHI	-16,184	-10,294	-19,367	-13,204	0
ODI	-10,204	-7,131	-13,061	-7,510	0
LowSpO2	5,633	5,066	3,592	7,857	0

For all parameters examined, bootstrap test was conducted to assess the homogeneity of the groups before and after the surgery, with a p-value > 0.05. Thus, for all parameters examined, show that there is no significant difference between the mean of the BRP and sBRP groups. Regarding the functional results, comparison of the mean of the AHI, ODI, and Lowest O_2 saturation parameters pre- and post-surgery in the two groups examined showed no statistically significant differences (two sample bootstrap t-test).

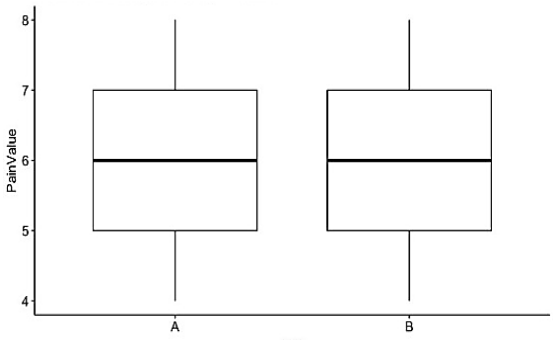


Figure 4. Pain Value Analysis between BRP group and sBRP group.

The results of the pain assessment analyzed at day 3 after surgery using a visual analog scale (VAS 0 -10, with 0 no pain, and 10 Worst imaginable post-operative pain) (Chiarotto et al. 2019; Williamson and Hoggart 2005) show no significant differences between the two study groups (Figure 4).
Only one patient in the standard BRP group had suture extrusion. No patient in the sBRP group had suture extrusion.

4. Discussion

The post-operative polygraphic measurements and the design of this clinical study seem to confirm one of the peculiar characteristics of BRP pharyngoplasty, that it is a procedure that allows the surgeon a certain freedom in its execution. Post-operative polygraphic data at 6

months confirm the efficacy of the technique even in the simplified form described.

We reconciled the conflicting demands of technique simplification with a modest impact on post- operative polygraphic outcomes by using Dual-Angle technology of wound closure Barbed device led to barbs with strong anchoring force. This technology was utilized using a unidirectional, single needle, Knotless, Tissue-Closure Device on each side of the pharynx.

The sBRP does not use the double-needle Barbed suture recommended for the standard BRP in order to couple the potential of dual-angle suture technology with the potential of using a single-needle unidirectional suture on each side of the pharynx: suturing the two sides of the pharynx separately allows a better management of calibration and balancing of pulling forces on soft tissues and this allows to exploit the full potential of dual-angle suture technology.

Separate management of the vector quantities applicable to the sidewalls, which is possible with the use of unidirectional/single-needle sutures, allows us greater freedom in the choice of force application points, which, in this study, do not appear to have adversely affected the functional results.

This separate handling of sidewall collapsibility can also compensate for asymmetries due to technique inaccuracies.

We wanted to eliminate the last step of the standard BRP procedure because in the context of a procedure that is essentially easy to learn, a critical moment for the surgeon performing BRP pharyngoplasty for the first time is the execution of the final step.

Elimination of step 8 also appears to be able to reduce one of the minor complications which is a parcel extrusion of the suture most frequently placed there.

With the elimination of the last step of the BRP, a fundamental vector is eliminated and replaced by an additional loop of reinforcement of the suture between Pterygomandibular Raphe and Palatopharyngeus Muscle. In this way, while fixing only the collapsible lateral pharyngeal wall to a stable structure such as the Pterygomandibular Raphe and eliminating vertical vectors, we had no negative impact on polygraphic outcomes at 6 months post-surgery. Subsequent studies will be performed to compensate for the lack of long-term patient follow-up and to increase the sample population size under study.

5. Conclusion

In cases of OSA from retropalatal obstruction, the sBRP shows functional results at 6 months superimposable compared to the BRP technique from which it derives while being even less invasive. The sBRP version described above is also easier to execute, which benefits its dissemination.

6. References

- Alessandri, Franco, Valentino Remorgida, Pier Luigi Venturini, and Simone Ferrero. 2010. "Unidirectional Barbed Suture versus Continuous Suture with Intracorporeal Knots in Laparoscopic Myomectomy: A Randomized Study." *Journal of Minimally Invasive Gynecology* 17 (6): 725–29. https://doi.org/10.1016/j.jmig.2010.06.007.
- Arigliani, Michele, Domenico M. Toraldo, Enrico Ciavolino, Caterina Lattante, Luana Conte, Serena Arima, Caterina Arigliani, Antonio Palumbo, and Michele De Benedetto. 2021. "The Use of Middle Latency Auditory Evoked Potentials (MLAEP) as Methodology for Evaluating Sedation Level in Propofol-Drug Induced Sleep Endoscopy (DISE) Procedure." *International Journal of Environmental Research and Public Health* 18 (4): 2070. https://doi.org/10.3390/ijerph18042070.
- Arigliani, Michele, Domenico M. Toraldo, Filippo Montevecchi, Luana Conte, Lorenzo Galasso, Filippo De Rosa, Caterina Lattante, et al. 2020. "A New Technological Advancement of the Drug-Induced Sleep Endoscopy (DISE) Procedure: The 'All in One Glance' Strategy." *International Journal of Environmental Research and Public Health* 17 (12): 4261. https://doi.org/10.3390/ijerph17124261.
- Berry, Richard B, Rohit Budhiraja, Daniel J Gottlieb, David Gozal, Conrad Iber, Vishesh K Kapur, Carole L Marcus, et al. 2012. "Rules for Scoring Respiratory Events in Sleep: Update of the 2007 AASM Manual for the Scoring of Sleep and Associated Events. Deliberations of the Sleep Apnea Definitions Task Force of the American Academy of Sleep Medicine." Journal of Clinical Sleep Medicine : *JCSM : Official Publication of the American Academy of Sleep Medicine* 8 (5): 597–619. https://doi.org/10.5664/jcsm.2172.
- Brandley, Efron, and J. Robert Tibshirani. 1993. An Introduction to the Bootstrap. *Edited by Chapman & Hall*. New York.
- Chiarotto, Alessandro, Lara J. Maxwell, Raymond W. Ostelo, Maarten Boers, Peter Tugwell, and Caroline B. Terwee. 2019. "Measurement Properties of Visual Analogue Scale, Numeric Rating Scale, and Pain

Severity Subscale of the Brief Pain Inventory in Patients With Low Back Pain: A Systematic Review." *The Journal of Pain* 20 (3): 245–63. https://doi.org/10.1016/j.jpain.2018.07.009.

- Dachuri, Sandeep, Ravi Sachidananda, Filippo Montevecchi, Mohamed Rashwan, Claudio Vicini, Sowjanya Gandla, Satish Kumaran Pugazhendi, and Srividya Rao Vasista. 2019. "Barbed Reposition Pharyngoplasty in Indian Population: A New Palatal Surgery for OSAS." *Indian Journal of Otolaryngology and Head & Neck Surgery* 71 (2): 249–53. https://doi.org/10.1007/s12070-018-1320-9.
- Georgalas, C., G. Garas, E. Hadjihannas, and A. Oostra. 2010. "Assessment of Obstruction Level and Selection of Patients for Obstructive Sleep Apnoea Surgery: An Evidence-Based Approach." Journal of Laryngology and Otology. *J Laryngol Otol*. https://doi.org/10.1017/S002221510999079X.
- Kotecha, Bhik, and Andrea De Vito. 2018. "Drug Induced Sleep Endoscopy: Its Role in Evaluation of the Upper Airway Obstruction and Patient Selection for Surgical and Nonsurgical Treatment." *Journal of Thoracic Disease. AME Publishing Company*. https://doi.org/10.21037/jtd.2017.10.32.
- Mantovani, M, A Minetti, S Torretta, A Pincherle, G Tassone, and L Pignataro. 2012. "The Velo-Uvulo-Pharyngeal Lift or 'Roman Blinds' Technique for Treatment of Snoring: A Preliminary Report." Acta Otorhinolaryngologica Italica : *Organo Ufficiale Della Societa Italiana Di Otorinolaringologia e Chirurgia Cervico-Facciale* 32 (1): 48–53.
- Montevecchi, F., G. Meccariello, E. Firinu, M. S. Rashwan, M. Arigliani, M. De Benedetto, A. Palumbo, et al. 2018. "Prospective Multicentre Study on Barbed Reposition Pharyngoplasty Standing Alone or as a Part of Multilevel Surgery for Sleep Apnoea." *Clinical Otolaryngology* 43 (2): 483–88. https://doi.org/10.1111/coa.13001.
- Rinaldi Vittorio, Mario Mantovani, and Lorenzo Pignataro. 2017. "Barbed Suture Rescue Procedure." *Aesthetic Surgery Journal* 37 (2): 250–52. https://doi.org/10.1093/asj/sjw096.
- Strollo, Patrick J., and Robert M. Rogers. 1996. "Obstructive Sleep Apnea." New England *Journal of Medicine* 334 (2): 99–104. https://doi.org/10.1056/NEJM199601113340207.
- Vicini, Claudio, Ehsan Hendawy, Aldo Campanini, Mohamed Eesa, Ahmed Bahgat, Saleh AlGhamdi, Giuseppe Meccariello, Andrea DeVito, Filippo Montevecchi, and Mario Mantovani. 2015. "Barbed Reposition Pharyngoplasty (BRP) for OSAHS: A Feasibility, Safety, Efficacy and Teachability Pilot Study. 'We Are on the Giant's Shoulders.'" *European Archives of Oto-Rhino-Laryngology* 272 (10): 3065–70. https://doi.org/10.1007/s00405-015-3628-3.
- Williamson, Amelia, and Barbara Hoggart. 2005. "Pain: A Review of Three Commonly Used Pain Rating Scales." *Journal of Clinical Nursing* 14 (7): 798–804. https://doi.org/10.1111/j.1365-2702.2005.01121.x.
- Yaggi, H. Klar, John Concato, Walter N. Kernan, Judith H. Lichtman, Lawrence M. Brass, and Vahid Mohsenin. 2005. "Obstructive Sleep Apnea as a Risk Factor for Stroke and Death." *New England Journal of Medicine* 353 (19): 2034–41. https://doi.org/10.1056/NEJMoa043104.

Journal of InterDisciplinary Research Applied to Medicine
JDREAM
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5 i. 1, 27-32
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p27
http://siba-ese.unisalento.it, © 2021 Università del Salento

Adenoid Cystic Carcinoma of the Bartholin Gland. A Morphological and Immunohistochemical study of a rare case

Alessandro D'Amuri[1], Federica Floccari[2], Marcello Filotico[3], Ottavia D'Oria[4,5], Gaetano Panese[4], Andrea Tinelli[4,6,7]

[1]Anatomic Pathology Unit, "A. Perrino" Hospital, Brindisi, Italy
[2]Clinic Pathology Unit, "L. Bonomo" Hospital, Andria (BT) – Italy
[3]Anatomic Pathology Unit, "G. Panico" Hospital, Tricase, Lecce, Italy
[4]Department of Obstetrics and Gynecology, "Veris delli Ponti" Hospital, Scorrano, Lecce, Italy
[5]Department of Medical and Surgical Sciences and Translational Medicine, PhD Course in "Translational Medicine and Oncology", Sapienza University, Rome, Italy
[6]Division of Experimental, Endoscopic Surgery, Imaging, Technology and Minimally Invasive Therapy, Vito Fazzi Hospital, Lecce, Italy
[7]Laboratory of Human Physiology, Phystech BioMed School, Faculty of Biological & Medical Physics, Moscow Institute of Physics and Technology (State University), Dolgoprudny, Moscow Region, Russia

Corresponding author: Andrea Tinelli
andreatinelli@gmail.com

Abstract

Adenoid cystic carcinoma of the Bartholin gland (BG-ACC) is a rare form of vulvar cancer. Literature reported approximately 350 cases of BG-ACC since 1864.
Literature data and case reports suggested an aggressive nature with protracted clinical symptoms and a tendency for local recurrence despite adequate surgical excision with or without adjuvant radiotherapy.
Survival rates of 71% and 59% are reported, respectively, at five and ten years.
A comparative analysis of the immunohistochemical profile was performed with the homologous tumor of the salivary glands, and it was observed that the expression of various antigen in different morphological patterns of this neoplasia allowed some considerations about on its histogenesis that was hitherto never proposed.

Keywords: Bartholin's gland cyst, adenoid cystic carcinoma, vulvar cancer, female genital tumor, immunohistochemistry

1. Introduction

The primary carcinoma of the Bartholin gland (BG) is a rare female genital tumor, accounting for less than 1% of all malignant tumors of the female genital tract. The rare frequency and the difficult macroscopic diagnosis, during a clinical evaluation, make it a mysterious object, given its rarity (Crum et al. 2014). The scientific literature reports very few cases studied and published, even if the ACC-BG at early stage can be treated by wide local excision as a primary surgery (Khan and Abbasi 2020). Most commonly, generally ACC-BG has a propensity for perineural invasion and is therefore associated with high local recurrence rates (Nieuwenhuyzen-de Boer et al. 2020). The wide local excision and radical vulvectomy with or without lymph node dissection, are performed (Khan and Abbasi 2020). More long-term follow up is recommended to evaluate optimal primary treatment and roles of radiotherapy and chemotherapy because ACC-BG recurs and metastasizes long after primary treatment (Şahin Aker, Cansız Ersöz, and Ortaç 2020). In our experience, authors report a case of adenoid cystic carcinoma of the Bartholin gland (BG-ACC) in their practice, discussing histopathology and its histogenesis.

2. Case Report

A 66-year-old woman, with no previous gynecological history, for about two years, presented a perineal painless swelling, corresponding with the posterior third of the left labium minus, con-

sidered in a first time as a Bartholin cyst. The patient was scheduled for surgical marsupialization. During perineal surgery, a solitary solid and mobile mass was noted in the BG, with lobulated margins of 30 x 27 mm, extending into subcutaneous and muscle tissue. An excisional biopsy was performed. The surgical sample was a nodular formation of 30 x 20 mm, with pink color, with a granular and shiny surface, of sustained consistency. The specimen was fixed in formalin and in paraffin embedded.

The sections stained with hematoxylin and eosin, and histochemical stains with PAS and Blu Alcian. The tissue was submitted to a panel of a several immunohistochemical antibodies: CKAE1/AE3, CK5/6, CK7, CK19, CK20, EMA, p63, SMACT, calponin, S-100, CD10, CD34, CD117, ER, PgR, mammaglobin, GCDFP-15, CEA, p53 and Ki67. The morphological finding of the lesion was detected as neoplastic. Lesion was largely represented by acinar structures of cribriform appearance, in which the holes were delimited by small, flattened cells (Figure 1a) and were occupied by a dense, weakly basophilic material (Figure 1b), PAS and Blue-Alcian positive (Figures 4a, 4b). Solid, nodular-looking cell agglomerates were scattered in mass samples. They consisted of small roundish, mononuclear cells with poor cytoplasm and a hyperchromatic nucleus (Figures 1c, 1d, 2a). Some of these agglomerates had an entirely solid appearance, while others showed a mixed aspect, in which the cribriform component was also represented in varying degrees. The latter started from the periphery of the nodule progressing, in a centripetal direction, until it was completely occupied (Figures 2d, 3a). Neoplastic proliferation also occurred in the form of tubular structures, delimited by a monolayer cylinder-cubic epithelium above a basal layer consisting of small-medium roundish mononuclear elements. This component, however, was completely minor, accounting for no more than 10% of the proliferation. It was also detected a diffuse perineural permeation and infiltration of periglandular structures (Figures 3b, 3c, 3d). The results of immunohistochemical research were in cribriform pattern (CKAE1/AE3 +, CK5/6 +, CK7 +, CK19 -/+, CK20 -, EMA +, CEA -, p63 +, SMACT +, S-100 + in neural structures, CD10 -, CD34 -, CD117 -, ER -, PgR - , p53 -/+ and Ki67 >5%), in solid pattern (CKAE1/AE3 +, CK5/6 -, CK7 -, CK19 -, CK20 -, EMA -/+, CEA -, p63 +, SMACT +, S-100 -, CD10 -, CD34 -, CD117 - , ER -, PgR - , p53 -/+ and Ki67 >5%), and in tubular pattern (CKAE1/AE3 +, CK5/6 + luminal, CK7 +, CK19 + luminal, CK20 + luminal, EMA -, CEA +, p63 +, SMACT +, S-100 -, CD10 -, CD34 -, CD117 - , ER -, PgR - , p53 -/+ and Ki67 >5%). The diagnosis of BG-ACC tumor, involving surgical margins with perineural invasion, was finally performed by pathologist. A radiological staging, by computer tomography (CT), scanning chest, abdomen, and pelvis, revealed no metastatic evidence. Patient underwent radical local vulvectomy with bilateral inguinal lymph node dissection. Final diagnosis was a FIGO Stage 1b vulvar cancer. Patient completed six cycles of adjuvant radiotherapy, 63 Gy/33 fractions to her vulva using VMAT (Vaginal Sparing with Volumetric Modulated Arc Therapy), and she remained disease-free at 9 months (the short follow up permitted to clinicians).

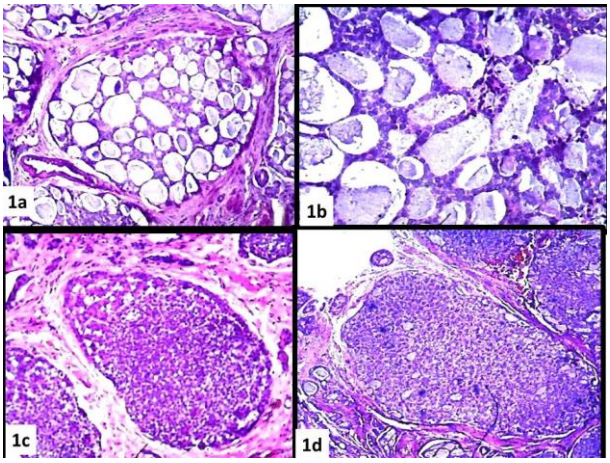


Figure 1. a) Acinar cribriform structure (HE); **b)** cribri containing inspissated basophilic material (HE); **c) d)** solid nodules

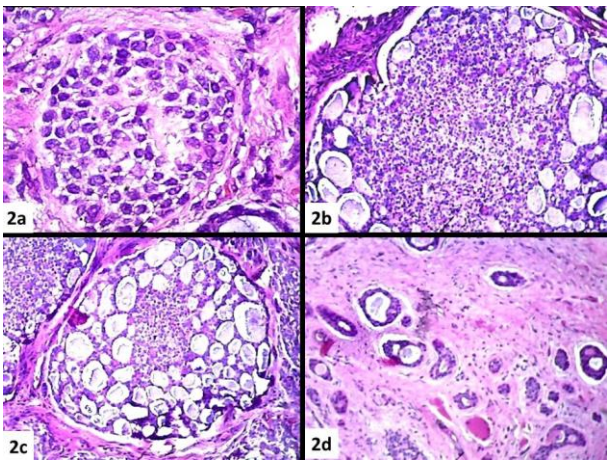


Figure 2. a) Solid nodule, particular (HE); **b) c)** solid nodules in acinar differentiation (HE); **d)** tubular structures (HE)

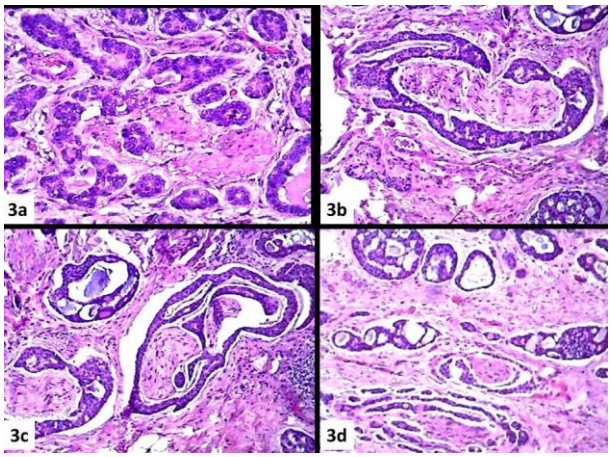


Figure 3. a) Tubular structures (HE); **b) c) d)** Infiltration of nervous trunks (HE).

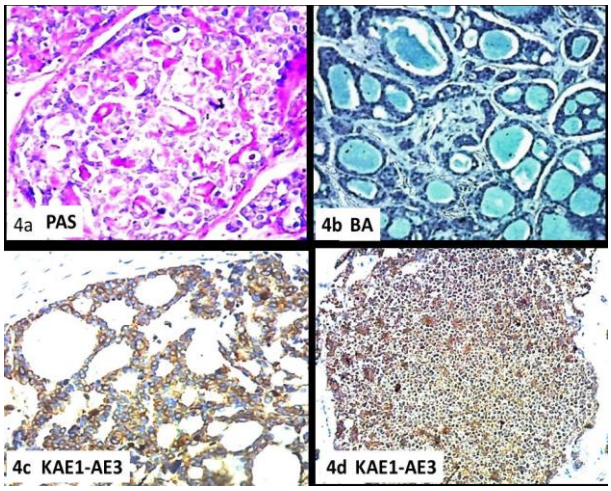


Figure 4. a) PAS **b)** Blu-Alcian **c) d)** Keratin AE1-AE3, cribriform acinus and solid nodule

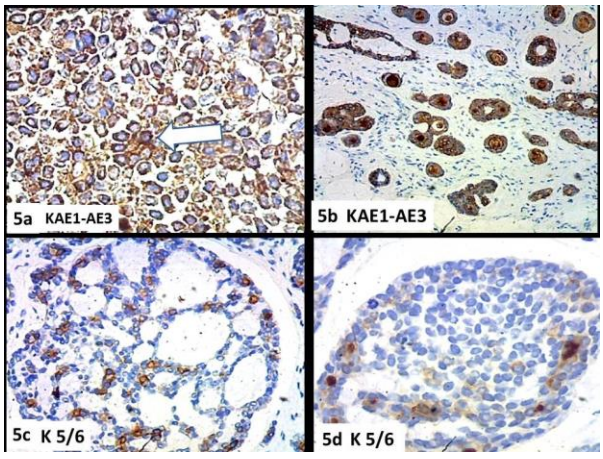


Figure 5 a) KAE1-AE3, floret-like cellular groups in a solid nodule; **b)** KAE1-AE3, tubular structures; **c) d)** K 5/6 in cribriform acinus and in solid nodule

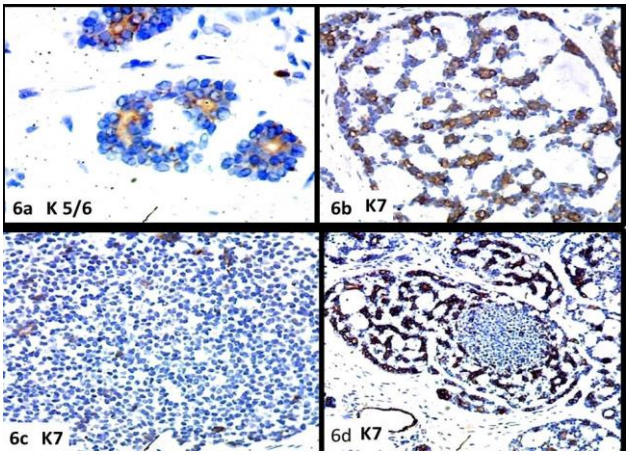


Figure 6. a) K 5/6 in tubular structures (luminal); **b) c) d)** K7 in a cribriform acinus, solid nodule, nodule undergoing differentiation

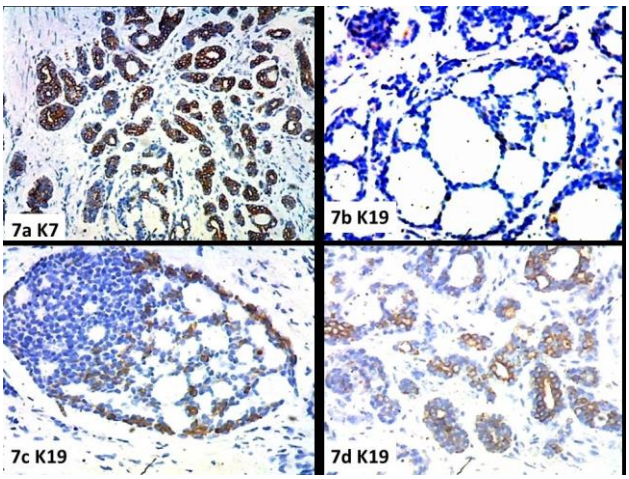


Figure 7. a) K7 Tubular structures; **b) c) d)** K19 in a cribriform acinus, solid nodule, tubular structures

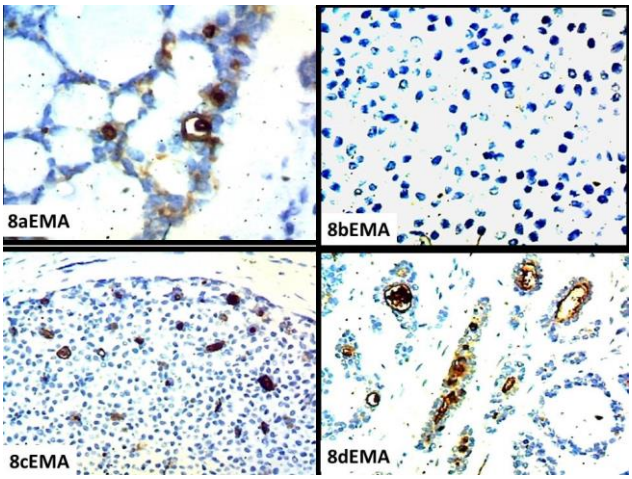


Figure 8. a) EMA a cribriform acinus; **b) c)** in a solid nodule, floret-like-cellular groups **d)** tubular structures (luminal)

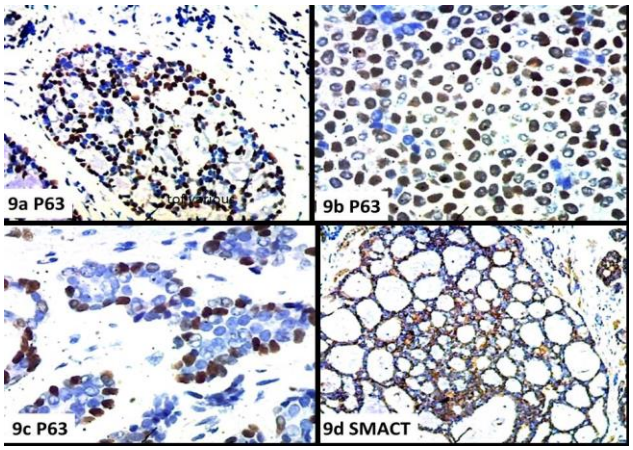


Figure 9. a) b) c) P63 in a cribriform acinus, solid nodule, tubular **d)** SMACT in a cribriform acinus

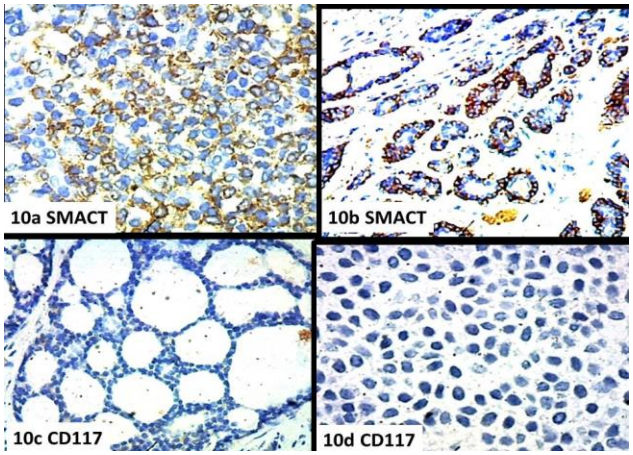


Figure 10. a) b) SMACT in a solid nodule and tubular structures **c) d)** CD117 in a cribriform acinus and in a solid nodule

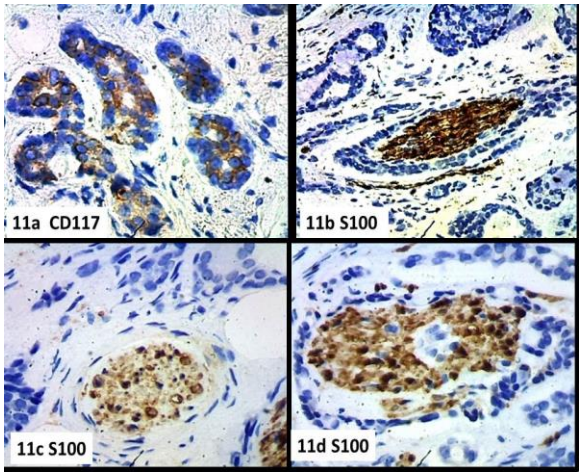


Figure 11. a) CD117 in tubular structures (luminal) **b) c) d)** S100, nerve trunks permeated by neoplastic proliferation

3. Discussion

In a study of 2011 (Alsan et al. 2011), 79 cases of ACC were recorded in literature, with an incidence of about 15% of all tumors of BG gland. Then, in a subsequent paper of 2017 (Di Donato et al. 2017), on 275 cases of primary tumors of BG 77 were ACC, with an incidence of about 30%. In this investigation, tumors were classified by histotype: 80 squamous cell carcinoma (30.7%), 77 ACC (29.6%), 65 adenocarcinoma (25%), 7 transitional cell carcinoma (2.6%), 7 sarcoma (2.6%), 3 neuroendocrine carcinoma (1.1%), 3 adenosquamous tumors (1.1%), 2 epithelioid-myoepithelial carcinoma (0.7%), 16 rare tumors as others (6.1%).

The ACC-BG accounts for only 0.30% of all female genital tract cancers, is considered as a rare malignancy and, therefore, worthy of further analysis and literature signaling. Like all BG neoplasms, the ACC has no specific perineal characteristics and is often clinically confused with the most frequent abscesses or cysts of the gland (Goh, McCully, and Wagner 2018). In the many literature reports, the most frequent sign during its perineal presentation was a vulvar mass in 147 cases (53.5%). Nevertheless, despite its indolent course, it has a high rate of local recurrence and hematogenous metastatization, especially to the lungs. A typical characteristic of this neoplasm is the perineural penetration, explaining the high recurrence rate, even after complete tumor excision (Anaf et al. 1999).

To better understand this rare neoplastic entity, it is opportune to discuss on the structure and immunohistochemical characteristics of Bartholin gland. The Bartholin gland contains three types of epithelium. The glandular acini are lined by mucinous columnar epithelium, emerging in the ducts with a transitional epithelium and becoming squamous epithelium at the ostia opening on the vestibule vaginalis. The different histological types of tumors present in BG would be linked to the cell line from which it would take origin. Squamous carcinomas would originate from squamous orificial cells, transitional carcinomas from transitional cells of the excretory duct, adenocarcinoma from acinar cells (Tsukahara et al. 1991).

More complex, instead, the histogenesis of the ACC like that of morphologically similar tumors frequently arising in other locations, such as the

salivary glands, in the first line, followed by upper respiratory tract, nasopharynx, breast, uterine cervix and brain (Tsukahara et al. 1991).
Authors hypothesized that such neoplasms may originate from the reserve cells present in the intercalated small ducts of Bartholin gland that may have the potential to differentiate into two cell types, myoepithelial and luminal cells. As already recorded by other authors, as in our case, this histotype presents three morphological variously intermingled patterns: 1) one acinar cribriform (Figure 1a, 1b, 1c, 1d); 2) one solid, consisting of small mononuclear cells with a poor cytoplasmic halo (Figures 2a, 2b, 2c, 2d); 3) another tubular, consisting of luminal cubic cylinder elements below, which a layer of basal cells of small volume is present (Figures 3a, 3b, 3c, 3d) (Tsukahara et al. 1991).
In the mentioned case, the three patterns are irregularly mixed, with a clear prevalence of that acinar/cribriform, so authors separately evaluated the immunohistochemical profile of the three patterns:
a) the acinar / cribriform pattern presented positivity for almost all the cytokeratins tested, even with varying intensities and distribution. CKAE1/AE3 intense and diffuse (Figures 4a, 4b, 4c, 4d), CK5/6 expressed by the cells located in the nodal points of the cribriform (Figure 5c), Ki67 intense and diffuse (Figure 6b), CK19 presented only in some acini in marginal place (Figure 7b) and EMA expressed in single elements in the nodal points of the cribri (Figure 8a), positive p63 (Figure 9a) and SMACT (Figure 9d), negatives S100, CD34, CD117, CEA, ER, PGR, p53, Ki67 <5%;
b) the solid pattern presented positivity for CKAE/AE3 (Figure 4d), p63 (Figure 9b) and SMACT (Figure 10a). Concerning to the nodules, scattered groups of larger elements in a floret-like arrangement were noticed, expressing cytokeratin more intensely (Figure 5a) and, additionally, these elements expressed EMA (Figures 8b, 8c). Transitional aspects were present in significant number, wherein the formation of the cribriform begins from the periphery of the nodules up to completely replace the solid component, with a subsequent expression of the different types of cytokeratin (Figure 6d).
c) the tubular pattern, the less represented in the proliferation, expressed almost all cytokeratins, except for CK20, with a diffuse character, the CKAE1-AE3 (Figure 5b) and 7 (Figure 7a), the 5/6 (luminal) (Figure 6a) and CK19 (luminal) (Figure 7d), EMA (luminal) (Figure 8d), p63 (Figure 8c) and SMACT (Figure 10b) (basal). In a similar vein, CD117 (luminal) was also positive (Figure 11a), S100 was positive only in the nervous trunks infiltrated by the neoplasm (Figures 11b, 11c, 11d).
An article on the BG tumors, reported for the ACC the following immunohistochemical profile: CKAE1/AE3 +, CK8/18 +, SMA +, SMM +, p63 +, S-100 + and EMA + (Goh, McCully, and Wagner 2018).
Overall, the immunophenotypic profile of our case is in line with the data reported in the literature. The expression of many antigens in different morphological patterns allowed us some histogenetic considerations hitherto never advanced. The "primum movens" would be identified in the solid nodules generated by the proliferation of elements with a morphologic and immunophenotypic myoepithelial profile. From the periphery of these nodules begins the morphological and immunohistochemical epithelial differentiation that leads to the formation of the cribriform acinar structures and to the tubular ones. In the above-mentioned case, there are evidence of the nerve structures permeation phenomena in surrounding tissues, that made the neoplasms prognosis very severe, as several times reported in literature.

4. Conclusions

The primary ACC-BG is a rare vulval cancer, characterized by slow growth, local invasion, and sometimes distant metastasis. Available literature suggests an aggressive nature with protracted clinical symptoms and local recurrence despite adequate surgical excision with or without adjuvant radiotherapy. Local recurrences are common and often precede distant metastases (Yoon et al. 2015). The recommended primary treatment is vulvectomy, obtaining clear margins and bilateral inguinofemoral node dissection. Despite being diagnosed at a more advanced stage, patients with primary carcinoma of the Bartholin gland seem to have similar oncologic outcomes and survival rates to patients

with non-Bartholin gland-related vulvar carcinoma (Zhan et al. 2014).

5. References

- Alsan, Cetin I, V Vinh-Hung, F Eren, and U Abacioğlu. (2011). "Adenoid Cystic Carcinoma of the Bartholin's Gland: Case Report and Systematic Review of the Literature." *European Journal of Gynaecological Oncology* 32 (5): 567–72.
- Anaf, V., F. Buxant, F. Rodesch, P. Simon, J. van de Stadt, J.- C. Noel, and J. van Geertruyden. (1999). "Adenoid Cystic Carcinoma of Bartholin's Gland:What Is the Optimal Approach?" *European Journal of Surgical Oncology (EJSO)* 25 (4): 406–9. https://doi.org/10.1053/ejso.1999.0666.
- Crum, CP, WG McCluggage, CS Herrington, S Regauer, and EJ Wilkinson. (2014). Tumours of the Vulva; Epithelial Tumors. Edited by Young RH Kurman RJ, Carcangiu ML, Herrington CS. *WHO Classification of Tumours of Female Reproductive Organs. Lyon: International Agency for Research on Cancer.*
- Donato, Violante Di, Assunta Casorelli, Erlisa Bardhi, Flaminia Vena, Claudia Marchetti, Ludovico Muzii, and Pierluigi Benedetti Panici. (2017). "Bartholin Gland Cancer." *Critical Reviews in Oncology/Hematology* 117, 1–11. https://doi.org/10.1016/j.critrevonc.2017.06.005.
- Goh, Siew Pei, Brian McCully, and Matthew Kyle Wagner. (2018). "A Case of Adenoid Cystic Carcinoma Mimicking a Bartholin Cyst and Literature Review." *Case Reports in Obstetrics and Gynecology* 1–3. https://doi.org/10.1155/2018/5256876.
- Khan, Rozilla Sadia, and Huda Saleem Abbasi. (2020). "A Different Strategy for Management of a Case of Adenoid Cystic Carcinoma of Bartholin Gland." JPMA. *The Journal of the Pakistan Medical Association* 70 (8): 1445–47. https://doi.org/10.5455/JPMA.29969.
- Nieuwenhuyzen-de Boer, Gatske M., Shatavisha Dasgupta, Patricia C. Ewing-Graham, and Mieke R. Van Bockstal. (2020). "Adenoid Cystic Carcinoma of the Bartholin Gland Is Not HPV-Related: A Case Report and Review of Literature." *Pathology - Research and Practice* 216 (6): 152968. https://doi.org/10.1016/j.prp.2020.152968.
- Şahin Aker, Seda, Cevriye Cansız Ersöz, and Fırat Ortaç. (2020). "Adenoid Cystic Carcinoma of Bartholin's Gland Diagnosed after Lung Lobectomy: Review of the Literature and a Case Presentation." *Journal of Turkish Society of Obstetric and Gynecology* 17 (4): 310–13. https://doi.org/10.4274/tjod.galenos.2020.39225.
- Tsukahara, Yoshiharu, Atsushi Mori, Toru Fukuta, Tsutomu Katsuyama, and Osamu Yamagami. (1991). "Adenoid Cystic Carcinoma of Bartholin&Rsquo;s Gland: A Clinical, Immunohistochemical and Ultrastructural Study of a Case with Regard to Its Histogenesis." *Gynecologic and Obstetric Investigation* 31 (2): 110–13. https://doi.org/10.1159/000293113.
- Yoon, Gun, Hyun-Soo Kim, Yoo-Young Lee, Tae-Joong Kim, Chel Hun Choi, Sang Yong Song, Byoung-Gie Kim, Duk-Soo Bae, and Jeong-Won Lee. (2015). "Analysis of Clinical Outcomes of Patients with Adenoid Cystic Carcinoma of Bartholin Glands." *International Journal of Clinical and Experimental Pathology* 8 (5): 5688–94.
- Zhan, Ping, Gang Li, Bin Liu, and Xi-guang Mao. (2014). "Bartholin Gland Carcinoma: A Case Report." *Oncology Letters* 8 (2): 849–51. https://doi.org/10.3892/ol.2014.2200.

Journal of InterDisciplinary Research Applied to Medicine
JDREAM
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5 i. 1, 33-36
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p33
http://siba-ese.unisalento.it, © 2021 Università del Salento

How is suspected transthyretin-related cardiac amyloidosis diagnosed? Role 99mTc-HDMP scintigraphy: a substitute for biopsy?

Alessandra Cimino[1], Elisa Rizza[1], Luca Luongo[1], Angelo Mita[1]

[1]Nuclear Medicine Unit, Interdisciplinary Department of Medicine. Lecce, Italy

Corresponding author: Alessandra Cimino
ale.cimi90@gmail.com

Abstract

Cardiac amyloidosis (CA) is characterized by extracellular deposition of protein-derived fibrils and lead to heart failure. Gold standard for its etiological diagnosis is endomyocardial biopsy and laboratory tests, both high-cost and invasive procedures. Technetium- 99m hydroxymethylene diphosphonate (99mTc-HMDP) scintigraphy is important tool for defining CA, specifically transthyretin subtype (ATTR). From July 2020 to February 2021, we retrospectively analyzed 18 pts [14 males, 4 females; aged 32-86y] with suspected ATTR, underwent to scintigraphy 150 min after iv administration of 740 MBq 99mTc-HMDP.Myocardial uptake was assessed optically based on Perugini Score (0- 3). Biopsy confirmed diagnosis. Intense (Score 3) and moderate (Score 2) myocardial uptake verified in 8 patients by 99mTc-HDMP scintigraphy, was consistent with ATTR suspect. In 10 patient's cardiac radiotracer uptake was absent (Score 0) avoiding biopsy. Our data indicate a 99mTc-HDMP scintigraphy key role in the early diagnosis but even more in the exclusion of patients with ATTR subtype, optimizing the management of pts who do not require high costs and invasive procedures.

Keywords: Cardiac Amyloidosis; ATTR amyloidosis; (99m)Tc-HMDP; Technetium-labeled bone scintigraphy; Cardiomyopathy.

1. Introduction

Systemic amyloidosis is heterogeneous disease characterized by extracellular deposition of protein-derived fibrils, namely amyloid, in different tissue and organs, including the heart.
Although considered a rare disease, recent data suggest that cardiac amyloidosis is underappreciated as a cause of common cardiac diseases or syndromes (Maleszewski 2015).
While more than 30 proteins are known to be capable of aggregating as amyloid in vivo, only nine amyloidogenic proteins accumulate in the myocardium to cause significant cardiac disease, like heart failure, conduction disorders, atrial fibrillation and ventricular arrhythmias.
Two types of amyloid commonly infiltrate the heart: immunoglobulin light-chain (AL) amyloid and transthyretin (ATTR) amyloid, either in its hereditary (ATTRv) or acquired (ATTRwt) form.
Cardiac amyloidosis is diagnosed when amyloid fibrils are found within cardiac tissue. (Benson et al. 2018).
Both invasive and non-invasive diagnostic criteria have been proposed.
Imaging with cardiac US/MRI provides nonspecific findings.
Gold standard for etiological diagnosis of cardiac amyloidosis is endomyocardial biopsy combined with immunohistochemical parameters/mass spectroscopy, both high-cost and invasive procedures.
Technetium- 99m hydroxymethylene diphosphonate (99mTc-HMDP) scintigraphy is a important tool for defining CA, specifically transthyretin subtype (ATTR). (Ruberg et al. 2019)
Our work underlines the role of nuclear medicine in CA diagnosis and patient's management.

2. Subjects and methods

2.1 Patients' population

We retrospectively analyzed eighteen patients [14 males, 4 females; aged 32-86y] who were admitted to our Unit from July 2020 to February 2021 with suspected CA.

The following variables were recorded for each patient: age, gender, hospitalization department, risk factors, symptoms, and previous clinical and instrumental evaluation.

CA suspicion was based on presence clinical cardiac and extracardiac sign and symptoms, like hypotension, macroglossia, skin bruises, carpal tunnel syndrome, polyneuropathy and dysautonomia, altered ECG results and laboratory tests, pathological instrumental imaging results (Echocardiogram and/ or cardiac magnetic resonance)

For each patient, an individual informed consensus was obtained allowing us to use all data for research purposes.

2.2 Diagnostic exams

Myocardial scintigraphy was acquired using gamma OPTIMA NM/CT 670 (GE Medical System, West Milwaukee, WI, USA) 150 minutes after iv administration of 740 MBq 99mTc- labelled hydroxymethylene diphosphonate (HMDP).

Whole -body planar images were acquired 3 hours after injection. Images were acquired with low-energy and high-resolution collimators and a scan speed of 10 cm/min.

Myocardial radiotracer uptake was assessed optically based on Perugini Score (Perugini et al. 2005):

0: absent of tracer myocardial uptake and normal bone uptake.

1: mild cardiac uptake, inferior to bon uptake.

2: moderate cardiac uptake with attenuated bone.

3: high cardiac uptake with decreased or absent bone uptake. (Figure 1) ECG test showed low/decreased QRS voltage to degree of LV thickness and/or atrio-ventricular conduction disease.

Persistent troponin elevation, disproportionally elevated NT-proBNP to degree of heart failure and proteinuria (even mild) in laboratory tests were also signs that could evoke CA.

All patients were performed Echocardiogram, characterized by presence of granular sparkling of myocardium, increased right ventricular wall/ valve thickness and pericardial effusion.

8/18 patients underwent cardiac magnetic resonance that showed subendocardial late gadolinium enhancement, elevated native T1 values, Increased extracellular volume and Abnormal gadolinium kinetics.

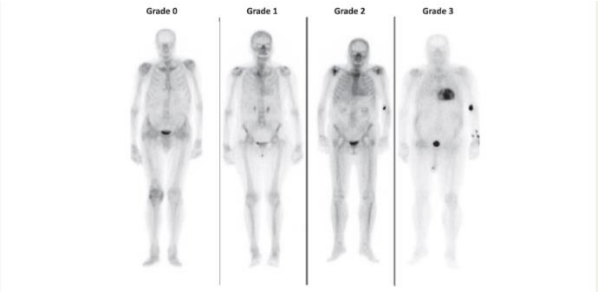


Figure 1. Cardiac uptake grading in bisphosphonate scintigraphy.

3. Results

Diffuse intense myocardial uptake (score 3) verified in 5 patients by 99mTc-HDMP scintigraphy. This result, associated with positive hematologic tests (serum free light chains and serum and urine immunofixation), was consistent with ATTR diagnosis, without biopsy.

In 3 patients whole-body scintigraphy showed a moderate cardiac uptake (Score 2), biopsy was necessary to confirm.

In ten patient's cardiac radiotracer uptake was absent (Score 0) and hematologic tests were negative, so biopsy invasive procedure was avoiding, according to diagnostic algorithm. (Figure 2)

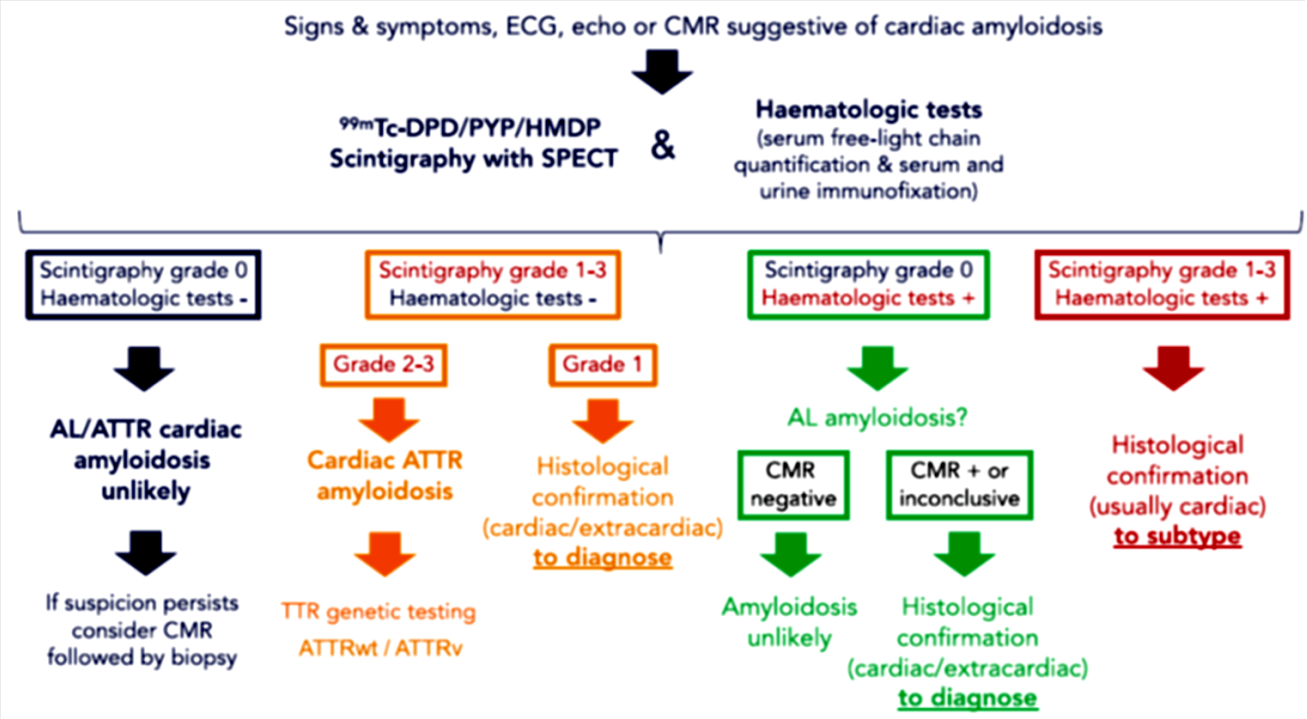


Figure 2 Diagnostic algorithm for cardiac amyloidosis. AL, light-chain amyloidosis;ATTR, transthyretin amyloidosis; ATTRv, hereditary transthyretin amyloidosis; ATTRwt, wild-type transthyretin amyloidosis; CMR, cardiac magnetic resonance; ECG, electrocardiogram; SPECT, single photon emission computed tomography; TTR, transthyretin.

4. Discussion

Cardiac amyloidosis is confirmed when an endomyocardial biopsy demonstrates amyloid deposits after Congo red staining irrespective of the degree of left ventricular (LV) wall thickness. Identification of amyloid should be followed by classification of the amyloid fibril protein (Gonzalez-Lopez et al. 2015).

Although the gold standard for defining the type of amyloid remains mass spectrometry, immunohistochemistry, or immunoelectron microscopy are routinely employed for amyloid typing in specialized centers (Maleszewski 2015)

Diagnosis is also confirmed if amyloid deposits within an extracardiac biopsy are accompanied either by characteristic features of cardiac amyloidosis by echocardiography, in the absence of an alternative cause for increased LV wall thickness, or by characteristic features on cardiac magnetic resonance (CMR).

99mTc-HDMP scintigraphy important role in diagnostic algorithm for cardiac amyloidosis: it's a simple, non-invasive, low-cost and widely available modality. It does not require preparation and has no side effect so it can be performed in all types of patients including hemodynamically complicated patients (Bokhari er al. 2013).

The availability of modern SPET/CT technology, as in our center, allows to obtain acquisition of tomographic images associated with morphology, thus increasing diagnostic accuracy of CA.

Detection of ATTR in our study population by 99mTc-HDMP scintigraphy was accomplished with 100% sensitivity and specificity (Castano et al. 2016).

A diagnostic algorithm based initially on the use of bone scintigraphy coupled to assessment for monoclonal proteins allows appropriate diagnosis in patients with suggestive signs/symptoms.

Early CA diagnosis, especially in ATTR, is necessary to establish correct therapy.

Infact, management of cardiac amyloidosis involves treatment and prevention of complications, and halting or delaying amyloid deposition

by specific treatments, including stabilizing molecules (tafamidis) and genetic silencers (patisiran and inotersen) for ATTR amyloidosis. (Rapezzi et al. 2021).

5. Conclusions

Our data indicate that 99mTc-HDMP scintigraphy has a key role in CA early diagnosis but even more in the exclusion of patients with the ATTR subtype.
99mTc-HDMP scintigraphy confirms to be a simple, non-invasive, low-cost and widely available modality. It does not require preparation and has no side effect so it can be performed in all types of patients including hemodynamically complicated patients.
This examination, optimizing the management of pts who do not require admission to procedures with high costs and more invasive, is useful for earlier diagnosis and screening of CA.

6. *References*

- Maleszewski. (2015). *"Cardiac amyloidosis: pathology, nomenclature, and typing."* Cardiovasc Pathol 24:343-350.
- Benson, Buxbaum, Eisenberg, Merlini, Saraiva, Sekijima, Sipe, and Westermark. (2018*). "Amyloid nomenclature 2018: recommendations by the International Society of Amyloidosis (ISA) nomenclature committee"* Amyloid. 25: 215–219.
- Ruberg, Grogan, Hanna, Kelly, and Maurer. (2019). *"Transthyretin amyloid cardiomyopathy: JACC state-of-the-art review."* J Am Coll Cardiol. 73: 2872–2891.
- Perugini, Guidalotti, Salvi, Cooke, Pettinato, Riva, Leone, Farsad, Ciliberti, Bacchi-Reggiani, Fallani, Branzi, and Rapezzi. (2005). *"Noninvasive etiological diagnosis of cardiac amiloidosis using 99mTc-3,3-diphosphono-1,-propanodicarboxylic acid scintigraphy."* J Am Coll Cardiol. 46 (6): 1076-1084.
- Gonzalez-Lopez, Gallego-Delgado, Guzzo-Merello, De Haro-Del Moral, Cobo-MArcos, Robles, Bornsterin, Salas, Lara-pezzi, Alfonso-Pulpon, and Garcia-Pavia. (2015*). " Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction"*. European Hear Journal. 36: 2585-94
- Bokhari, Castaño, Pozniakoff, Pozniakoff, Deslisle, Latif, and Maurer. (2013*). "99mTc-pyrophosphate scintigraphy for differentiating light chain cardiac amyloidosis from the transthyretin-related familial and senile cardiac amyloidoses"*. Circ Cardiovasc Imaging. 6 (2): 195-201.
- Castano, Haq, Narotsky, Goldsmith, Weinberg, Morgenstern, Pozniakoff, Ruberg, Miller, Berk, Dispenzieri, Grogan, Johnson, Bokhari, and Maurer. (2016). *"Multicenter experience of planar technetium pyrophosphate cardiac imaging: Does preferential cardiac uptake predict survival in patients with ATTR cardiac amyloidosis?"* JAMA Cardiol. 1 (8): 880-889.
- Rapezzi, Elliott, Damy, Nativi-Nicolau, Berk, Velazquez, Boman, Gundapaneni, Patterson, Schwartz, Sultan, and Maurer. (2021). *"Efficacy of tafamidis in patients with hereditary and wild-type transthyretin amyloid cardiomyopathy: further analyses from ATTR-ACT"*. JACC Heart Fail. 9 (2) :115–123.

Journal of InterDisciplinary Research Applied to Medicine
JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5, i. 1, 37-48
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p37
http://siba-ese.unisalento.it, © 2021 Università del Salento

The rhythms of language: an overview of linguistic processes and neural oscillations

Cosimo Iaia[1], Barbara Gili Fivela[1], Francesco Sigona[1], Mirko Grimaldi [1]

[1]Department of Humanities & CRIL-DReAM, University of Salento, Italy

Corresponding author: Cosimo Iaia
cosimo.iaia@studenti.unisalento.it

Abstract

For the last decades neuroscientists have grown interest in the analysis of the rhythmic activity of the brain synchronized at temporal and spatial level. These neural oscillations, grouped by their frequency, have been proposed to govern all cognitive processes. In the field of the neurobiology of language, considerable research has linked speech processing and language comprehension to neural oscillations. On one hand, neural rhythmic activity is thought to synchronize to relevant spectral information of speech on three-time scales – which physically reflect phoneme, syllable and phrase processing. On the other hand, syntactic and semantic processing is subserved by faster oscillatory patterns not necessarily related to the acoustic properties of speech. For each linguistic process, this article summarizes the neural oscillations involved. Further evidence comes from studies on language-related pathologies.

Keywords: brain rhythms, linguistic operations, speech processing, language comprehension

1. *Introduction*

Neuroscientists using magnetoencephalography (MEG) and electroencephalography (EEG) have greatly relied on event-related potentials (ERPs) and event-related magnetic fields (ERFs) to investigate the major components involved in linguistic processes – a positive or negative deflection of the signal in respect of a baseline – such as P100, N100, P200, P300, N400, P600 (Swaab et al. 2012). Thereby, considerable research has tested the involvement of distinct brain regions and the concomitant electrical/magnetic activity for various linguistic processes, highlighting the spatiotemporal distribution of neural activation. This approach is based on the idea that each stimulus (visual, auditory and so on) a subject is exposed may elicit a time-locked neural response. However, this response cannot be seen in the raw EEG signal, due to the overlapping of ongoing background activity. To determine these systematic responses, a certain experimental paradigm is repeated a number of times: each time a stimulus is presented to the subject, a marker is set into EEG/ERF signal to obtain time-locked epochs to the experimental event of interest: that is, the components previous cited. By averaging all the epochs, only the systematic response should remain (Sauseng and Klimesch 2008).

Recent advancement in neurolinguistic research have seen a shift in paradigm: a central question has become not only which brain region is responsible for which function but also how brain regions interact with each other. In fact, it is necessary to explore "not only what is connected, but how and in what directions regions of the brain are connected" (Kopell et al. 2014, 1319) by adding a functional perspective to understand how the brain's regions are involved in producing and processing brain signals (Murphy 2015).

Although ERPs/ERFs have proved to be extremely useful, this approach overshadows that the EEG activity of the human brain is not flat and that functions, especially complex ones, involve different areas. In fact, all the electrical activity recorded at the scalp is characterized by

rhythms, which are driven by fluctuations in excitability of large-sized populations of neurons, with specific spatiotemporal patterns that differ in amplitude, timing, and frequency (Cohen 2017). Depending on their frequency, such rhythms are grouped in delta (δ: ~0.5–4 Hz), theta (θ: ~4–8 Hz), alpha (α: ~8–12 Hz), beta (β: ~12–30 Hz) and gamma (γ: ~30–120 Hz). Neural oscillations have found wide use in clinical applications, providing useful information about levels of consciousness, psychological states, or presence of neurological disorders.

Recently, researchers have come to realize that these oscillatory rhythms also subserve a wide variety of cognitive processes: it has been argued that the synchronization and desynchronization of these oscillations in distinct clusters can shape input gain and assist information transfer (Akam and Kullmann 2010; Muller et al. 2018). In fact, strong evidence suggests that the reorganization of ongoing oscillatory patterns might explain some of the features of ERPs/ERFs, due to phase reset (i.e., the reshaping of the signal) once a stimulus is presented to the subject (Başar et al. 2001; Başar 2011). Therefore, event-related oscillations, further than to have the time-locked EEG information, permits the retrieval of non-phase locked EEG information related to the cognitive activity induced by the stimulus

As for language, neural oscillations have been linked to a number of linguistic operations. This article provides an overview of neural oscillations subserving linguistic operations. Following Meyer (2018), a dichotomy between lower-level functions of speech processing and higher-level functions of language comprehension will be assumed: on one hand, linguistically meaningful units must be segmented from speech, based on temporal and spectral cues recognized by the auditory system; on the other hand, two streams of language comprehension are assumed to occur to decode the meaning of words (semantic stream) and the relations between words (syntactic stream).

2. *The delta-(theta-gamma) neural code for speech pro- cessing*

2.1. *Speech processing on three timescales*

By paraphrase of Meyer (2018), the segmentation and identification of discrete phonological units have been found to occur in a particular range of operational frequencies. Phonological units decreasing in granularity hierarchically build speech: the combination of phonemes result into syllables; the combination of syllables result into intonation phrases. Each phonological unit has an acoustics-temporal counterpart (Gussenhoven and Jacobs 2017).

In the last decade, researchers have found that neural oscillations might subserve a set of neural operations that allows the segmentation and identification of discrete phonological units. In fact, during speech processing, three frequency bands, gamma, theta and delta bands, seem to synchronize respectively with the pace of phonemes, syllables and intonational phrases, by tracking linguistically meaningful acoustic properties of speech on three different time scales (Bourguignon et al. 2020; Giraud and Poeppel 2012; Molinaro and Lizarazu 2018). The synchronization of neural oscillations to speech is thought to occur thanks to the so-called neural entrainment which relies on phase synchronization and amplitude synchronization (Obleser and Kayser 2019): on one hand, bottom-up modulations of neural oscillations are stimulus-dependent, relying on acoustic properties of speech; on the other hand, neural oscillations have been found to internally organize, building hierarchical structures, where lower-frequency bands top-down modulate higher-frequency bands, regardless of stimulus properties (Fontolan et al. 2014).

2.2. *Stimulus-bound processing*

Starting with bottom-up modulations, the phonemic time scale falls within the gamma band frequencies (30-120 Hz). As discussed by Meyer (2018), low and high gamma band oscillations may subserve acoustic and categorical processing, respectively: low gamma-band phase synchronization seems to be related to acoustic processing (Gross et al. 2013), while phonemic-categorical perception is subserved by amplitude synchronization of high gamma bands (Lehongre et al. 2011), which reflect the spiking activity of neurons in the auditory cortex sensitive to phonemes (Mesgarani et al. 2014). In addition, it has been argued that low gamma band synchronization occurs more

strongly with the acoustic amplitude envelope compared to phonemic-categorical information (Di Liberto, O'Sullivan, and Lalor 2015).
Going up in granularity, theta bands oscillations (4-8 Hz) capture the pace of syllables, thus subserving syllabic processing. In fact, recent works have suggested that theta oscillations phase-synchronize to the onset of syllables, allowing the segmentation of syllables (Luo and Poeppel 2007; Howard and Poeppel 2012; Peelle, Gross, and Davis 2013; Doelling et al. 2014; references from Meyer 2018). Moreover, further evidence suggests a relation- ship between the amplitude modulations of speech and the phase of neural oscillations (Gross et al. 2013; Vander Ghinst et al. 2016; Molinaro, Monsalve, and Lizarazu 2016).
Lastly, delta bands (0.5-4 Hz) have shown increased phase coherence to the fundamental frequency envelope of speech: delta oscillations have been proposed to aid the segmentation of intonational phrases (Giraud and Poeppel 2012), due to the amplitude extrema of the pitch contour marking the boundaries of intonational phrases. Interestingly, delta bands also capture the pace of syntactic phrases (Ding et al. 2016; Molinaro and Lizarazu 2018), which do not have a direct physical counterpart, in the case prosodic cues were explicitly removed (Ding et al. 2017). However, the role of delta oscillations in speech processing is still under debate (see for example Boucher, Gilbert, and Jemel 2019).

2.3. *Top-down modulations*

While strong evidence suggests bottom-up modulations of neural oscillations, it has been argued that these oscillations hierarchically self-organize, regardless of acoustic properties of speech: particularly, the phase of lower-frequency bands top-down modulates the amplitude of higher-frequency bands (Giraud and Poeppel 2012; Fontolan et al. 2014), opening a new window on brain dynamics of speech processing. In fact, theta-gamma cross frequency coupling in the left hemisphere have been proposed to subserve the concatenation of phonemes into syllables (Canolty et al. 2006), although this hypothesis contrasts with a number of studies that show a theta-gamma coupling in the right auditory cortex (Luo and Poeppel 2007; Abrams et al. 2008; Hämäläinen et al. 2012; Gross et al. 2013; Howard and Poeppel 2012; Peelle, Gross, and Davis 2013; references from Meyer 2018). Interestingly, it has been shown that phase-amplitude coupling between theta and gamma oscillations adapts to speech rate (Lizarazu, Lallier, and Molinaro 2019). The combination of syllables into intonational phrases is then subserved by delta-theta cross-frequency coupling (Giraud and Poeppel 2012; Ding et al. 2016). It is worth noticing that the neural underpinnings of prosody are still not clear and need future research (for a discussion see Myers, Lense, and Gordon 2019), given that, at least in some cases, prosody conveys crucial information on the syntactic structure which suggests a tight relation with top-down information.

3. *Language comprehension along two streams*

3.1 Syntactic processing

Once all phonological units are segmented from speech and the auditory system is tempo- rarily aligned, the brain must decode the rela- tions between words which are recursively combined into syntactic phrases (Chomsky 1957). Recent evidence suggests that the group- ing of words into phrases might be subserved by delta band cycles through phase resetting. In fact, Ding and colleagues (2016) have found an increase in delta-power associated with internal syntactic phrase generation. These findings were later confirmed by Bonhage et al. (2017): subjects involved in this study showed an in- crease in delta band power while exposed to a list of words that could be combined into syn- tactic phrases, while a decrease in delta band power was found for a list of words that could not be grouped into phrases. As mentioned above, delta band oscillations also play a role in the segmentation of intonational phrases. Interestingly, these results may not contradict each other: in fact, Ghitza (2017) argued that the relation between delta bands and intonational phrases would reflect a stimulus-bound bottom-up segmentation, while delta oscillations subserving syntactic chunking would reflect top-down generation based on a priori syntactic knowledge. However, the role of delta bands in syntactic phrase generation is still under debate. While a sentence unfolds word by word, phrases have to be stored in verbal working

memory and retrieved later on to assess their dependencies with other phrases and generate syntactic hierarchies. A number of studies have linked the storage of phrases in verbal working memory with an increase in alpha band activity (Haarmann and Cameron 2005; Weiss et al. 2005; Meyer, Obleser, and Friederici 2013; Bonhage et al. 2017; references from Meyer 2018). Particularly, alpha band power increases with storage demands, local- ized in the inferior parietal cortex.

Interesting findings come from violation studies that examined time-locked neural oscillations related to syntactic anomalies. Many of these studies have found a consistent pattern in response to syntactic violations, such as gender and number agreement violation (Bastiaansen, van Berkum, and Hagoort 2002; Davidson and Indefrey 2007; Schneider et al. 2016), mostly confirming the aforementioned findings.

A groundbreaking result concerns the possible role of gamma band in structure-building operations, what generative linguists call *Merge*: (Nelson and colleagues (2017) found a specific gamma band pattern that they claim as evidence for Merge, the binding. Particularly, gamma power increases every time a new word is added to an unfolding sentence, while it sharply decreases when words can be compressed into a syntactic node. In addition, a recent study has shown a difference in high gamma response for the syntactic disambiguation of homophones phrases (Artoni et al. 2020). However, these results may be in contrast with aforementioned studies linking delta activity to syntactic processing.

3.2. Semantic processing

Along the syntactic parsing, language comprehension also implies a semantic processing which has been linked primarily with beta and gamma oscillations. Evidence of beta-bands involvement in semantic processing have come from a number of violation studies, focused on semantic anomalies (Kielar et al. 2014; 2015; Wang et al. 2012a; Luo et al. 2010). Particularly, these studies have found a decrease both in alpha and beta oscillations related to semantic anomalies. Willems, Oostenveld, and Hagoort (2008) have linked both alpha and beta decrease to audio-visual semantic anomalies. However, they found that alpha activity decreases where both a visual and linguistic context mismatch occurs.

Interestingly, other research on semantic anomalies has also found an increase in theta power (Hagoort et al. 2004; Hald, Bastiaansen, and Hagoort 2006; Davidson and Indefrey 2007; M. Bastiaansen and Hagoort 2015; Wang, Zhu, and Bastiaansen 2012; references from Prystauka and Lewis 2019). Bastiaansen, Mazaheri, and Jensen (2012) proposed that theta power increase due to semantic anomalies might reflect the integration of the anomalous word into the sentence. Another interesting proposal comes from Prystauka and Lewis (2019): given that theta increase has also been found in syntactic violation studies and has been proposed to aid lexical-semantic retrieval (Bastiaansen, Mazaheri, and Jensen 2012; Marko, Cimrová, and Riečanský 2019), an increase in theta power may reflect a general error detection mechanism. This idea is also supported by other studies on incorrect solutions to mathematical equations (Tzur and Berger 2007) or motor error in reaching a task (Arrighi et al. 2016).

However, violation studies do not give the full picture: semantic processing also relies on predictions of upcoming words. Each word is stored in the long-term memory with a certain probability of occurring in a given context, prior and after other words (Hagoort et al. 2004; Kutas and Federmeier 2010). Top-down predictions, independent of stimuli, have also been linked with beta bands power which increases when expectations of upcoming words are confirmed and decreases when such predictions do not match the sequence of incoming words (Lewis and Bastiaansen 2015; Lewis et al. 2016). For example, Wang et al. (2012) performed a cloze test, finding a beta power decrease in sentence ending that did not matched expectations. These findings were further confirmed by Lewis et al. (2017) that compared short stories of sentences, observing an increase in beta power in semantically coherent stories and a decrease in beta power for semantically incongruent stories. An interesting proposal about the role of beta bands and prediction comes from Lewis et al. (2016), yielding that they might subserve predictions across different linguistic levels, from the auditory domain to the syntactic level (Kim and Chung 2008; Sabine Weiss and Mueller 2012; Arnal, Wyart, and Giraud 2011; Arnal and Giraud 2012). However, Meyer

(2018) argues that beta bands only subserve lexical-semantic predictions for two reasons: beta bands power increase during contextual prediction of upcoming words correlates with the amplitude of N400, indicating the lexical-semantic predictability of a word (Kutas and Federmeier 2010; Wang et al. 2012; Hale 2016; Lewis et al. 2016) but not its syntactic category (Levy 2008; Frank et al. 2015); beta-bands have been shown to be modulated by syntactic factors only in syntactic violation studies (e.g.: the syntactic category of the upcoming words do not match expectations), possibly yielding that semantic processing does not occur when syntactic parsing is not accomplished (Steinhauer and Drury 2012).

Alongside top-down predictions based on the occurrence frequency of each word in context stored in long-term memory, lexical-semantic representations of incoming words must be checked. When lexical-semantic predictions are fulfilled, gamma power has been found to increase (Wang, Zhu, and Bastiaansen 2012; Molinaro, Barraza, and Carreiras 2013; Monsalve, Pérez, and Molinaro 2014). Conversely, gamma power decreases when the incoming word does not match expectations (Hald, Bastiaansen, and Hagoort 2006; Penolazzi, Angrilli, and Job 2009; Rommers, Dijkstra, and Bastiaansen 2012; references from Meyer 2018).

The interplay between beta and gamma bands has been included in the predictive coding framework (e.g. Friston 2005). In fact, data collected on beta and gamma bands are compatible with the predictive coding framework (Lewis and Bastiaansen 2015; for a discussion see Meyer 2018; Prystauka and Lewis 2019).

4. *Language-related disorders and neural oscillations*

Further evidence of the implications of neural oscillations into linguistic operations comes from research on language-related pathologies and disorders. In fact, a number of studies has confirmed the aforementioned findings on linguistic operations and neural oscillations.

Current models of aphasia classifications still rely on the Wernicke-Lichtheim model which links damages in a brain area with a specific function. This model has the advantage of being simple: for example, a damage in the motor area of language, Broca's area, will be linked to a non-fluent aphasic syndrome, while damage in the sensory area of language, Wernicke's ar- ea, will be linked to fluent aphasia syndrome (Lichteim 1885; Wernicke 1974).

A number of studies have observed an alteration of neural oscillations both at resting state and while performing a linguistic task. Spironelli and Angrilli (2009), for example, demonstrated that an increase in delta amplitude in the perilesional area is a marker of brain damage in chronic non-fluent aphasic patients. In fact, delta band might be an index of neural inhibition. Other research has shown that focal lesions in the left hemispheric language regions may lead to a change in brain physiology. For example, Meinzer et al. (2004) found an increase in spontaneous delta activity in the perilesional area in a group of stroke patients suffering from different aphasia types, while also reporting a decrease in spontaneous perilesional delta activity after an intense speech and language therapy. Dubovik et al. (2012) also found a shift from fast to slow spontaneous neural oscillations, particularly in delta and theta frequency range. Interestingly, Nicolo et al. (2015) reported that more coherent beta oscillations in lesioned Broca's area in early post stroke recovery patients predicts future language improvement during recovery.

Functional restoration of the brain in post-stroke patients seems to be related to an increase in spontaneous alpha-band synchronization (Westlake et al. 2012; Dubovik et al. 2012). Moreover, Kielar et al. (2016) investigated the functional reorganization of language networks: particularly, in a group of subjects suffering from different types of aphasia, they found a decrease in alpha and beta power in the left hemisphere, where the lesion occurred, in response to semantic anomalies during sentence comprehension.

A previous study also reported a possible role of beta activity as an index of the reorganization of language networks in aphasic patients: Spironelli, Manfredi, and Angrilli (2013) reported that non-fluent aphasic subjects, after linguistic recovery, showed a reduced beta activation in the core damaged area during a phonological and semantic task, while also showing an increased delta activity compared to healthy control subjects. They also found an increased high beta-activity in the left anterior sites during

the phonological and orthographic task. The authors of the study have interpreted these findings as an index of the reorganization of language in recovered non-fluent aphasic subjects at the left prefrontal sites.
In addition, many studies on dyslexic subjects have confirmed the role of neural oscillation into linguistic operations. For example, Lehongre et al. (2011) linked a decreased entrainment of the lower gamma band to phonological deficits, while Leong and Goswami (2014) suggested that rhythmic entrainment at the syllabic timescale is disrupted in dyslexic subjects. These results were recently confirmed by Lizarazu et al. (2021), proving an impairment of cortical entrainment in the delta and theta range to speech in dyslexic subjects.

7. *Conclusions*

Although this article is far from offering an exhaustive overview, it is clear that neural oscillations provide a new window on brain dynamics related to linguistic operations. The number of studies following the oscillation-based framework has been growing in the last decade, yielding an increasing interest in brain's oscillatory nature. On one hand, speech processing seems to be subserved by delta, theta and gamma bands, respectively at phrase, syllable and phoneme timescales. On the other hand, language comprehension is subserved by a variety of frequency bands involved in syntactic and semantic processing, including more general cognitive functions such as the implication of short- and long-term memory.
Bottom-up and top-down modulations of neural oscillations may provide a neural code for linguistic operations: the cyclicity of oscillatory rhythms' synchronization and desynchronization may represent a neural coding (and decoding) that matches linguistic computations, shortening the gap between broader neuroscientific investigations and more fine-grained linguistic investigations (Granularity Mismatch Problem, (Embick and Poeppel 2015). Accordingly, formal proposals of hierarchical organization of neural oscillations have emerged (Murphy 2019; Grimaldi 2019).

8. *References*

- Abrams, D. A., T. Nicol, S. Zecker, and N. Kraus. (2008). "Right-Hemisphere Auditory Cortex Is Dominant for Coding Syllable Patterns in Speech." *Journal of Neuroscience* 28 (15): 3958–65. https://doi.org/10.1523/JNEUROSCI.0187-08.2008.
- Akam, Thomas, and Dimitri M. Kullmann. (2010). "Oscillations and Filtering Networks Support Flexible Routing of Information." *Neuron* 67 (2): 308–20. https://doi.org/10.1016/j.neuron.2010.06.019.
- Arnal, Luc H., and Anne-Lise Giraud. (2012). "Cortical Oscillations and Sensory Predictions." *Trends in Cognitive Sciences* 16 (7): 390–98. https://doi.org/10.1016/j.tics.2012.05.003.
- Arnal, Luc H, Valentin Wyart, and Anne-Lise Giraud. (2011). "Transitions in Neural Oscillations Reflect Prediction Errors Generated in Audiovisual Speech." *Nature Neuroscience* 14 (6): 797–801. https://doi.org/10.1038/nn.2810.
- Arrighi, Pieranna, Luca Bonfiglio, Fabrizio Minichilli, Nicoletta Cantore, Maria Chiara Carboncini, Emily Piccotti, Bruno Rossi, and Paolo Andre. (2016). "EEG Theta Dynamics within Frontal and Parietal Cortices for Error Processing during Reaching Movements in a Prism Adaptation Study Altering Visuo-Motor Predictive Planning." Edited by Luigi Cattaneo. *PLOS ONE* 11 (3): e0150265. https://doi.org/10.1371/journal.pone.0150265 .
- Artoni, Fiorenzo, Piergiorgio d'Orio, Eleonora Catricalà, Francesca Conca, Franco Bottoni, Veronica Pelliccia, Ivana Sartori, et al. (2020). "High Gamma Response Tracks Different Syntactic Structures in Homophonous Phrases." *Scientific Reports* 10 (1): 7537. https://doi.org/10.1038/s41598-020-64375-9.
- Başar, Erol. (2011). *Brain-Body-Mind in the Nebu- lous Cartesian System: A Holistic Approach by Oscil- lations*. New York, NY: Springer New York. https://doi.org/10.1007/978-1-4419-6136-5.
- Başar, Erol, Canan Başar-Eroglu, Sirel Karakaş, and Martin Schürmann. (2001). "Gamma, Alpha, Delta, and Theta Oscillations Govern Cognitive Processes." *International Journal of Psychophysiology* 39 (2–3): 241–48. https://doi.org/10.1016/S0167-8760(00)00145-8.
- Bastiaansen, Marcel, Jos J.A. van Berkum, and Peter Hagoort. (2002). "Event-Related Theta Power Increases in the Human EEG during Online Sentence Processing." *Neuroscience Let- ters* 323 (1): 13–16.

https://doi.org/10.1016/S0304-3940(01)02535-6.

- Bastiaansen, Marcel, and Peter Hagoort. (2015). "Frequency-Based Segregation of Syn- tactic and Semantic Unification during Online Sentence Level Language Comprehension." *Journal of Cognitive Neuroscience* 27 (11): 2095– 2107. https://doi.org/10.1162/jocn_a_00829.
- Bastiaansen, Marcel, Ali Mazaheri, and Ole Jensen. (2012). "Beyond ERP's: Oscillatory Neuronal Dynamics." In *Oxford Handbook of Event-Related Potential Components*, 40.
- Bonhage, Corinna E., Lars Meyer, Thomas Gruber, Angela D. Friederici, and Jutta L. Mueller. (2017). "Oscillatory EEG Dynamics Underlying Automatic Chunking during Sentence Processing." *NeuroImage* 152 (May): 647– 57. https://doi.org/10.1016/j.neuroimage.2017.03.018.
- Boucher, Victor J., Annie C. Gilbert, and Boutheina Jemel. (2019). "The Role of Low-Frequency Neural Oscillations in Speech Processing: Revisiting Delta Entrainment." *Journal of Cognitive Neuroscience* 31 (8): 1205–15. https://doi.org/10.1162/jocn_a_01410.
- Bourguignon, Mathieu, Nicola Molinaro, Mikel Lizarazu, Samu Taulu, Veikko Jousmäki, Marie Lallier, Manuel Carreiras, and Xavier De Tiège. (2020). "Neocortical Activity Tracks the Hierarchical Linguistic Structures of Self-Produced Speech during Reading Aloud." *NeuroImage* 216: 116788. https://doi.org/10.1016/j.neuroimage.2020.116788.
- Canolty, R. T., E. Edwards, S. S. Dalal, M. Soltani, S. S. Nagarajan, H. E. Kirsch, M. S. Berger, N. M. Barbaro, and R. T. Knight. (2006). "High Gamma Power Is Phase-Locked to Theta Oscillations in Human Neocortex." *Science* 313 (5793): 1626–28. https://doi.org/10.1126/science.1128115.
- Chomsky, Noam. (1957). *Syntactic Structures*. Berlin: De Gruyter.
- Cohen, Michael X. (2017). "Where Does EEG Come From and What Does It Mean?" *Trends in Neurosciences* 40 (4): 208–18. https://doi.org/10.1016/j.tins.2017.02.004.
- Davidson, D.J., and P. Indefrey. (2007). "An Inverse Relation between Event-Related and Time–Frequency Violation Responses in Sentence Processing." *Brain Research* 1158 (July): 81–92. https://doi.org/10.1016/j.brainres.2007.04.082.
- Di Liberto, Giovanni M., James A. O'Sullivan, and Edmund C. Lalor. (2015). "Low-Frequency Cortical Entrainment to Speech Reflects Phoneme-Level Processing." *Current Biol- ogy* 25 (19): 2457–65. https://doi.org/10.1016/j.cub.2015.08.030.
- Ding, Nai, Lucia Melloni, Aotian Yang, Yu Wang, Wen Zhang, and David Poeppel. (2017). "Characterizing Neural Entrainment to Hierarchical Linguistic Units Using Electroencephalography (EEG)." *Frontiers in Human Neuroscience* 11 (September): 481. https://doi.org/10.3389/fnhum.2017.00481.
- Ding, Nai, Lucia Melloni, Hang Zhang, Xing Tian, and David Poeppel. (2016). "Cortical Tracking of Hierarchical Linguistic Structures in Connected Speech." *Nature Neuroscience* 19 (1): 158–64. https://doi.org/10.1038/nn.4186.
- Doelling, Keith B., Luc H. Arnal, Oded Ghitza, and David Poeppel. (2014). "Acoustic Landmarks Drive Delta–Theta Oscillations to Enable Speech Comprehension by Facilitating Perceptual Parsing." *NeuroImage* 85 (January): 761–68. https://doi.org/10.1016/j.neuroimage.2013.06.035.
- Dubovik, Sviatlana, Jean-Michel Pignat, Radek Ptak, Tatiana Aboulafia, Lara Allet, Nicole Gillabert, Cécile Magnin (2012). "The Behavioral Significance of Coherent Resting-State Oscillations after Stroke." *NeuroImage* 61 (1): 249–57. https://doi.org/10.1016/j.neuroimage.2012.03.024.
- Embick, David, and David Poeppel. (2015). "Towards a Computational(Ist) Neurobiology of Language: Correlational, Integrated and Explanatory Neurolinguistics." *Language, Cognition and Neuroscience* 30 (4): 357–66. https://doi.org/10.1080/23273798.2014.980750.
- Fontolan, L., B. Morillon, C. Liegeois-Chauvel, and Anne-Lise Giraud. (2014). "The Contribution of Frequency-Specific Activity to Hierarchical Information Processing in the Human Auditory Cortex." *Nature Communications* 5 (1): 4694. https://doi.org/10.1038/ncomms5694.
- Frank, Stefan L., Leun J. Otten, Giulia Galli, and Gabriella Vigliocco. (2015). "The ERP Response to the Amount of Information Conveyed by Words in Sentences." *Brain and Language* 140 (January): 1–11. https://doi.org/10.1016/j.bandl.2014.10.006.
- Friston, Karl. (2005). "A Theory of Cortical Responses." *Philosophical Transactions of the Royal*

Society B: Biological Sciences 360 (1456): 815–36. https://doi.org/10.1098/rstb.2005.1622.

- Ghitza, Oded. (2017). "Acoustic-Driven Delta Rhythms as Prosodic Markers." *Language, Cognition and Neuroscience* 32 (5): 545–61. https://doi.org/10.1080/23273798.2016.1232419.
- Giraud, Anne-Lise, and David Poeppel. (2012). "Cortical Oscillations and Speech Processing: Emerging Computational Principles and Operations." *Nature Neuroscience* 15 (4): 511–17. https://doi.org/10.1038/nn.3063.
- Grimaldi, Mirko. (2019). "From Brain Noise to Syntactic Structures: A Formal Proposal within the Oscillatory Rhythms Perspective." In *Linguistic Variation: Structure and Interpretation* edited by Ludovico Franco and Paolo Lorusso, 293–316. De Gruyter Mouton. https://doi.org/10.1515/9781501505201-017.
- Gross, Joachim, Nienke Hoogenboom, Gregor Thut, Philippe Schyns, Stefano Panzeri, Pascal Belin, and Simon Garrod. (2013). "Speech Rhythms and Multiplexed Oscillatory Sensory Coding in the Human Brain." Edited by David Poeppel. *PLoS Biology* 11 (12): e1001752. https://doi.org/10.1371/journal.pbio.1001752.
- Gussenhoven, Carlos, and Haike Jacobs. (2017). *Understanding Phonology*. Fourth edition. Understanding Language Series. London; New York: Routledge, Taylor & Francis Group.
- Haarmann, H, and K Cameron. (2005). "Active Maintenance of Sentence Meaning in Working Memory: Evidence from EEG Coherences." *International Journal of Psychophysiology* 57 (2): 115–28. https://doi.org/10.1016/j.ijpsycho.2005.03.017.
- Hagoort, Peter, Lea Hald, Marcel Bastiaansen, and Karl Magnus Petersson. (2004). "Integration of Word Meaning and World Knowledge in Language Comprehension." *Science* 304 (5669): 438. https://doi.org/10.1126/science.1095455.
- Hald, Lea A., Marcel C.M. Bastiaansen, and Peter Hagoort. (2006). "EEG Theta and Gamma Responses to Semantic Violations in Online Sentence Processing." *Brain and Language* 96 (1): 90–105. https://doi.org/10.1016/j.bandl.2005.06.007.
- Hale, John. (2016). "Information-Theoretical Complexity Metrics." *Language and Linguistics Compass* 10 (9): 397–412. https://doi.org/10.1111/lnc3.12196.
- Hämäläinen, Jarmo A., André Rupp, Fruzsina Soltész, Denes Szücs, and Usha Goswami. 2012. "Reduced Phase Locking to Slow Amplitude Modulation in Adults with Dyslexia: An MEG Study." *NeuroImage* 59 (3): 2952–61. https://doi.org/10.1016/j.neuroimage.2011.09.075.
- Howard, Mary F., and David Poeppel. (2012). "The Neuromagnetic Response to Spoken Sentences: Co-Modulation of Theta Band Amplitude and Phase." *NeuroImage* 60 (4): 2118– 27. https://doi.org/10.1016/j.neuroimage.2012.02.028.
- Kielar, Aneta, Tiffany Deschamps, Regina Jokel, and Jed A. Meltzer. (2016). "Functional Reorganization of Language Networks for Semantics and Syntax in Chronic Stroke: Evidence from MEG." *Human Brain Mapping* 37 (8): 2869–93. https://doi.org/10.1002/hbm.23212.
- Kielar, Aneta, Jed A. Meltzer, Sylvain Moreno, Claude Alain, and Ellen Bialystok. (2014). "Oscillatory Responses to Semantic and Syntactic Violations." *Journal of Cognitive Neuroscience* 26 (12): 2840–62. https://doi.org/10.1162/jocn_a_00670.
- Kielar, Aneta, Lilia Panamsky, Kira A. Links, and Jed A. Meltzer. (2015). "Localization of Electrophysiological Responses to Semantic and Syntactic Anomalies in Language Comprehension with MEG." *NeuroImage* 105 (January): 507–24. https://doi.org/10.1016/j.neuroimage.2014.11.016.
- Kim, June Sic, and Chun Kee Chung. (2008). "Language Lateralization Using MEG Beta Frequency Desynchronization during Auditory Oddball Stimulation with One-Syllable Words." *NeuroImage* 42 (4): 1499–1507. https://doi.org/10.1016/j.neuroimage.2008.06.001.
- Kopell, Nancy J., Howard J. Gritton, Miles A. Whittington, and Mark A. Kramer. (2014). "Beyond the Connectome: The Dynome." *Neuron* 83 (6): 1319–28. https://doi.org/10.1016/j.neuron.2014.08.016.
- Kutas, Marta, and Kara D. Federmeier. (2010). "Thirty Years and Counting: Finding Meaning in the N400 Component of the Event-Related Brain Potential (ERP)." *Annual Review of Psychology* 62 (1): 621–47. https://doi.org/10.1146/annurev.psych.093008.131123.

- Lehongre, Katia, Franck Ramus, Nadège Villiermet, Denis Schwartz, and Anne-Lise Giraud. (2011). "Altered Low-Gamma Sampling in Auditory Cortex Accounts for the Three Main Facets of Dyslexia." *Neuron* 72 (6): 1080– 90. https://doi.org/10.1016/j.neuron.2011.11.002.
- Leong, Victoria, and Usha Goswami. (2014). "Assessment of Rhythmic Entrainment at Multiple Timescales in Dyslexia: Evidence for Disruption to Syllable Timing." *Music: A Window into the Hearing Brain* 308 (February): 141–61. https://doi.org/10.1016/j.heares.2013.07.015.
- Levy, Roger. (2008). "Expectation-Based Syntactic Comprehension." *Cognition* 106 (3): 1126–77. https://doi.org/10.1016/j.cognition.2007.05.006.
- Lewis, Ashley G., and Marcel Bastiaansen. (2015). "A Predictive Coding Framework for Rapid Neural Dynamics during Sentence-Level Language Comprehension." *Cortex* 68 (July): 155–68. https://doi.org/10.1016/j.cortex.2015.02.014.
- Lewis, Ashley G., Jan-Mathijs Schoffelen, Herbert Schriefers, and Marcel Bastiaansen. (2016). "A Predictive Coding Perspective on Beta Oscillations during Sentence-Level Language Comprehension." *Frontiers in Human Neurosci- ence* 10 (March). https://doi.org/10.3389/fnhum.2016.00085.
- Lewis, Ashley G., Jan-Mathijs Schoffelen, Christian Hoffmann, Marcel Bastiaansen, and Herbert Schriefers. (2017). "Discourse-Level Semantic Coherence Influences Beta Oscillato- ry Dynamics and the N400 during Sentence Comprehension." *Language, Cognition and Neuro- science* 32 (5): 601–17. https://doi.org/10.1080/23273798.2016.1211300.
- Lichteim, L. (1885). "On Aphasia1." *Brain* 7 (4): 433–84. https://doi.org/10.1093/brain/7.4.433.
- Lizarazu, Mikel, Marie Lallier, Mathieu Bourguignon, Manuel Carreiras, and Nicola Molinaro. 2021. "Impaired Neural Response to Speech Edges in Dyslexia." *Cortex* 135 (Febru- ary): 207–18. https://doi.org/10.1016/j.cortex.2020.09.033..
- Lizarazu, Mikel, Marie Lallier, and Nicola Molinaro. (2019). "Phase–amplitude Coupling between Theta and Gamma Oscillations Adapts to Speech Rate." *Annals of the New York Acade- my of Sciences* 1453 (1): 140–52. https://doi.org/10.1111/nyas.14099.
- Luo, Huan, and David Poeppel. (2007). "Phase Patterns of Neuronal Responses Reliably Discriminate Speech in Human Auditory Cortex," *Neuron* 54 (6): 1001–10. https://doi.org/10.1016/j.neuron.2007.06.004.
- Luo, Y., Y. Zhang, X. Feng, and X. Zhou. 2010. "Electroencephalogram Oscillations Differentiate Semantic and Prosodic Processes during Sentence Reading." *Neuroscience* 169 (2): 654–64. https://doi.org/10.1016/j.neuroscience.2010.05.032.
- Marko, Martin, Barbora Cimrová, and Igor Riečanský. 2019. "Neural Theta Oscillations Support Semantic Memory Retrieval." *Scientific Reports* 9 (1): 17667. https://doi.org/10.1038/s41598-019-53813-y.
- Meinzer, Marcus, Thomas Elbert, Christian Wienbruch, Daniela Djundja, Gabriela Barthel, and Brigitte Rockstroh. 2004. "Intensive Language Training Enhances Brain Plasticity in Chronic Aphasia." *BMC Biology* 2 (1): 20. https://doi.org/10.1186/1741-7007-2-20.
- Mesgarani, N., C. Cheung, K. Johnson, and E. F. Chang. 2014. "Phonetic Feature Encoding in Human Superior Temporal Gyrus." *Science* 343 (6174): 1006–10. https://doi.org/10.1126/science.1245994.
- Meyer, Lars. 2018. "The Neural Oscillations of Speech Processing and Language Comprehension: State of the Art and Emerging Mechanisms." *European Journal of Neuroscience* 48 (7): 2609–21. https://doi.org/10.1111/ejn.13748.
- Meyer, Lars, Jonas Obleser, and Angela D. Friederici. 2013. "Left Parietal Alpha Enhancement during Working Memory-Intensive Sentence Processing." *Cortex* 49 (3): 711–21. https://doi.org/10.1016/j.cortex.2012.03.006.
- Molinaro, Nicola, Paulo Barraza, and Manuel Carreiras. 2013. "Long-Range Neural Synchronization Supports Fast and Efficient Reading: EEG Correlates of Processing Expected Words in Sentences." *NeuroImage* 72 (May): 120–32. https://doi.org/10.1016/j.neuroimage.2013.01.031.
- Molinaro, Nicola, and Mikel Lizarazu. 2018. "Delta(but Not Theta)-Band Cortical Entrainment Involves Speech-Specific Processing." *European Journal of Neuroscience* 48 (7): 2642–50. https://doi.org/10.1111/ejn.13811.
- Molinaro, Nicola, Irene F. Monsalve, and Mikel Lizarazu. (2016). "Is There a Common Oscillatory Brain Mechanism for Producing and Predicting Language?" *Language, Cognition*

and Neuroscience 31 (1): 145–58. https://doi.org/10.1080/23273798.2015.1077978.

- Monsalve, Irene F., Alejandro Pérez, and Nico- la Molinaro. (2014). "Item Parameters Dissoci- ate between Expectation Formats: A Regres- sion Analysis of Time-Frequency Decomposed EEG Data." *Frontiers in Psychology* 5 (August). https://doi.org/10.3389/fpsyg.2014.00847.
- Muller, Lyle, Frédéric Chavane, John Reynolds, and Terrence J. Sejnowski. (2018). "Cortical Travelling Waves: Mechanisms and Computational Principles." *Nature Reviews Neuroscience* 19 (5): 255–68. https://doi.org/10.1038/nrn.2018.20.
- Murphy, Elliot. (2015). "The Brain Dynamics of Linguistic Computation." *Frontiers in Psychol- ogy* 6 (October). https://doi.org/10.3389/fpsyg.2015.01515.
- ———. 2019. "Interfaces (Traveling Oscillations) + Recursion (Delta-Theta Code) = Language." In , 251–69.
- Myers, Brett, Miriam Lense, and Reyna Gordon. (2019). "Pushing the Envelope: Developments in Neural Entrainment to Speech and the Biological Underpinnings of Prosody Perception." *Brain Sciences* 9 (3): 70. https://doi.org/10.3390/brainsci9030070.
- Nelson, Matthew J., Imen El Karoui, Kristof Giber, Xiaofang Yang, Laurent Cohen, Hilda Koopman, Sydney S. Cash, et al. (2017). "Neurophysiological Dynamics of Phrase-Structure Building during Sentence Processing." *Proceed- ings of the National Academy of Sciences* 114 (18): E3669–78. https://doi.org/10.1073/pnas.1701590114.
- Nicolo, Pierre, Sviatlana Rizk, Cécile Magnin, Marie Di Pietro, Armin Schnider, and Adrian G. Guggisberg. (2015). "Coherent Neural Oscillations Predict Future Motor and Language Improvement after Stroke." *Brain* 138 (10): 3048–60. https://doi.org/10.1093/brain/awv200.
- Obleser, Jonas, and Christoph Kayser. (2019). "Neural Entrainment and Attentional Selection in the Listening Brain." *Trends in Cognitive Scienc- es* 23 (11): 913–26. https://doi.org/10.1016/j.tics.2019.08.004.
- Peelle, J. E., J. Gross, and M. H. Davis. (2013). "Phase-Locked Responses to Speech in Human Auditory Cortex Are Enhanced During Comprehension." *Cerebral Cortex* 23 (6): 1378–87. https://doi.org/10.1093/cercor/bhs118.
- Penolazzi, Barbara, Alessandro Angrilli, and Remo Job. (2009). "Gamma EEG Activity Induced by Semantic Violation during Sentence Reading." *Neuroscience Letters* 465 (1): 74–78. https://doi.org/10.1016/j.neulet.2009.08.065.
- Prystauka, Yanina, and Ashley Glen Lewis. (2019). "The Power of Neural Oscillations to Inform Sentence Comprehension: A Linguistic Perspective." *Language and Linguistics Compass* 13 (9). https://doi.org/10.1111/lnc3.12347.
- Rommers, Joost, Ton Dijkstra, and Marcel Bastiaansen. (2012). "Context-Dependent Semantic Processing in the Human Brain: Evidence from Idiom Comprehension." *Journal of Cognitive Neuroscience* 25 (December). https://doi.org/10.1162/jocn_a_00337.
- Sauseng, Paul, and Wolfgang Klimesch. (2008). "What Does Phase Information of Oscillatory Brain Activity Tell Us about Cognitive Processes?" *Neuroscience & Biobehavioral Reviews* 32 (5): 1001–13. https://doi.org/10.1016/j.neubiorev.2008.03.014.
- Schneider, Julie M., Alyson D. Abel, Diane A. Ogiela, Anna E. Middleton, and Mandy J. Maguire. (2016). "Developmental Differences in Beta and Theta Power during Sentence Processing." *Developmental Cognitive Neuroscience* 19 (June): 19–30. https://doi.org/10.1016/j.dcn.2016.01.001.
- Spironelli, Chiara, and Alessandro Angrilli. (2009). "EEG Delta Band as a Marker of Brain Damage in Aphasic Patients after Recovery of Language." *Neuropsychologia* 47 (4): 988–94. https://doi.org/10.1016/j.neuropsychologia.2008.10.019.
- Spironelli, Chiara, Mirella Manfredi, and Alessandro Angrilli. (2013). "Beta EEG Band: A Measure of Functional Brain Damage and Language Reorganization in Aphasic Patients after Recovery." *Cortex* 49 (10): 2650–60. https://doi.org/10.1016/j.cortex.2013.05.003.
- Steinhauer, Karsten, and John E. Drury. (2012). "On the Early Left-Anterior Negativity (ELAN) in Syntax Studies." *Brain and Language* 120 (2): 135–62. https://doi.org/10.1016/j.bandl.2011.07.001.
- Swaab, Tamara Y., Kerry Ledoux, C.C. Camblin, and Megan Boudewyn. (2012). "Language-Related ERP Components." *Oxford Handbook of Event-Related Potential Components*, January, 397–440. https://doi.org/10.1093/oxfordhb/9780195374148.013.0197.
- Tzur, Gabriel, and Andrea Berger. (2007). "When Things Look Wrong: Theta Activity in Rule Violation." *Neuropsychologia* 45 (13): 3122–

26. https://doi.org/10.1016/j.neuropsychologia.2007.05.004.

- Vander Ghinst, Marc, Mathieu Bourguignon, Marc Op de Beeck, Vincent Wens, Brice Marty, Sergio Hassid, Georges Choufani, et al. (2016). "Left Superior Temporal Gyrus Is Coupled to Attended Speech in a Cocktail- Party Auditory Scene." *The Journal of Neurosci- ence* 36 (5): 1596–1606. https://doi.org/10.1523/JNEUROSCI.1730-15.2016.
- Wang, Lin, Ole Jensen, Danielle van den Brink, Nienke Weder, Jan-Mathijs Schoffelen, Lilla Magyari, Peter Hagoort, and Marcel Bastiaansen. (2012). "Beta Oscillations Relate to the N400m during Language Comprehension." *Human Brain Mapping* 33 (12): 2898–2912. https://doi.org/10.1002/hbm.21410.
- Wang, Lin, Zude Zhu, and Marcel Bastiaansen. (2012). "Integration or Predictability? A Further Specification of the Functional Role of Gamma Oscillations in Language Comprehension." *Frontiers in Psychology* 3. https://doi.org/10.3389/fpsyg.2012.00187.
- Weiss, S, H Mueller, B Schack, J King, M Kutas, and P Rappelsberger. (2005). "Increased Neuronal Communication Accompanying Sentence Comprehension." *International Journal of Psychophysiology* 57 (2): 129–41. https://doi.org/10.1016/j.ijpsycho.2005.03.013.
- Weiss, Sabine, and Horst M. Mueller. (2012). ""Too Many Betas Do Not Spoil the Broth': The Role of Beta Brain Oscillations in Language Processing." *Frontiers in Psychology* 3. https://doi.org/10.3389/fpsyg.2012.00201.
- Wernicke, Carl. (1974). "Der Aphasische Symptomenkomplex." In *Der Aphasische Symptomencomplex*, 1–70. Springer.
- Westlake, Kelly P., Leighton B. Hinkley, Mon- ica Bucci, Adrian G. Guggisberg, Anne M. Findlay, Roland G. Henry, Srikantan S. Naga- rajan, and Nancy Byl. (2012). "Resting State Alpha-Band Functional Connectivity and Re- covery after Stroke." *Experimental Neurology* 237 (1): 160–69. https://doi.org/10.1016/j.expneurol.2012.06.020.
- Willems, Roel M., Robert Oostenveld, and Peter Hagoort. (2008). "Early Decreases in Alpha and Gamma Band Power Distinguish Linguistic from Visual Information during Spoken Sentence Comprehension." *Brain Research* 1219 (July): 78–90. https://doi.org/10.1016/j.brainres.2008.04.065.

JDREAM. Journal of interDisciplinary REsearch Applied to Medicine
JDREAM (2021), v. 5, i. 1, 49-59
ISSN 2532-7518
DOI 10.1285/i25327518v5i1p49
http://siba-ese.unisalento.it, © 2021 Università del Salento

Attitudes, perceptions, and knowledge of the population on End-of-Life and Advance Treatment Declaration: an observational study in Southern Italy

Antonino Calabro[1], Roberto Lupo[2], Ilaria Bernardini[3], Ornella De Mitri[4], Cosimo Caldararo[5], Marcello Antonazzo[6], Carmen Donadio[7], Maicol Carvello[8], Federica Ilari[9], Maria Chiara Carriero[10], Luana Conte[11-12]

[1]"Nuovo Ospedale degli Infermi" Hospital, ASL (Local Health Authority) Biella, Italy
[2]"San Giuseppe da Copertino" Hospital, ASL (Local Health Authority) Lecce, Italy
[3]"Antonio Perrino" Hospital, ASL (Local Health Authority) Brindisi, Italy
[4]"V.Fazzi" Hospital, ASL (Local Health Authority) Lecce, Italy
[5]"A. Moro" University, Bari, Italy
[6]"V. Fazzi" Hospital, ASL (Local Health Authority) Lecce. Italy
[7]"Salvatore Peragine" Hospital, ASL (Local Health Authority) Stigliano, Italy
[8]"Brisighella Community Hospital, ASL (Local Health Authority) Romagna, Brisghella, Ravenna, Italy.
[9]"University of Piemonte Orientale Biella, Italy
[10]"Santa Chiara Institute, Rome, Italy.
[11]Laboratory of Interdisciplinary Research Applied to Medicine (DReAM), University of Salento and ASL (Local Health Authority) Lecce, Italy
[12]Laboratory of Biomedical Physics and Environment, Department of Mathematics and Physics, University of Salento, Lecce, Italy.

Corresponding author: Luana Conte
luana.conte@unisalento.it

Abstract

In an advanced scientific and technological context, where it is now tangible the possibility of interfering indefinitely in the process of dying, it becomes necessary to disseminate knowledge about end of life that, for the great variety of areas that it invests, presents many controversial aspects. With the *Law no. 219/2017*, the right of self-determination and freedom of treatment of the patient is enshrined, aspects that to date still remain too little discussed. An online survey was conducted from December 2019 to February 2020, among the population residing in the provinces of Lecce and Brindisi, spread thanks to the collaboration of local authorities. A large part of the sample (82.4%, N=333) claims the right to self-determination, stating that therapeutic decisions are up to the patient who has signed his advance treatment dispositions, declared absolutely binding for 50% (N=205) of the sample. However, there is still a lack of information about how to draw up advance treatment agreements (AADs). 12.6% (N=51) of those interviewed stated that they knew nothing about it and only 32.9% (N=133) felt ready and adequately informed to make their own declarations. Another peculiar aspect is that topics such as euthanasia and assisted suicide seem to be considered at the margins of acceptability among End-of-Life instances. The results of the study show that knowledge on the subject has definitely improved over the years and that in most of the issues addressed, the population has an adequate degree of preparedness even though there is still some skepticism in dealing with issues such as assisted suicide and euthanasia. Future research could explore the possibility of identifying effective training tools and communication strategies that can be used by the widest possible segment of the population.

Keywords: End of life, Self-determination, Training, Information, Advance directive on treatment

1. Introduction

In Europe, and more generally in the Western world, the demographic transition has led to a prolongation of average life expectancy that has, however, coincided with a steady increase in chronic degenerative diseases (Riccioni et al. 2016). At the same time, in the health field, we are witnessing an impressive/impervasive scientific and technological progress which has allowed death to be transformed from a natural "event" into a medicalized "process"(Carlet et al. 2004; Lippo et al. 2014). In this context, in contemporary society, the most debated and controversial ethical issues are located, related to the end of life of the terminally ill person suffering from irreversible diseases, in conditions of discomfort and suffering. Moreover, as made evident by the dramatic case law cases presented in recent years, from Piergiorgio Welby to Eluana Englaro and Dj Fabo, have opened the political, social, and ethical debate on the end of life. Stories that have divided Italy, between those in favor and those against. The recognition of the right to refuse treatment as a correlate "*in negative*" of the principle of consent, has not prevented the emergence of various legal problems (Colacino et al. 2015), from the qualification of the interruption of medical treatment when it requires a phenomenologically active conduct, to the problem of representation and relevance of the will of the patient in a state of unconsciousness, which, in the continuing absence of legislative solutions, have been addressed by case law through the application of constitutional principles. The issues at the center of the discussion concern the limitation of care, therapeutic overkill, palliative care, advance declarations of treatment - DAT *(Law no. 219/2017)*, medically assisted suicide, euthanasia; terms, meanings and contents that are often not distinct, thwarted by the excessive spectacularizing/emphasizing/media distortion that empty them of the complexity, depth, and morality with which they are imbued. To engage in a discussion on the issues of Life, Death and Suffering is to highlight the fundamental bioethical principles and consider the diversity of views that arise from the analysis of them (Beauchamp et al. 2001). *Law no. 219/2017* ("Rules on informed consent and advance treatment dispositions") recognizes everyone is right to know their health conditions and to be informed in a complete and comprehensible way not only about the diagnosis, prognosis and benefits and risks associated with health checks and treatments, but also regarding the alternatives and consequences of any refusal of health treatment. Refusal of health treatment is part of the freedom of self-determination in health care, a freedom with respect to which the issue of the "end of life" emerges. The discipline on the rights of the person in end-of-life decisions, as also indicated in *Law no. 219/2017 (art.1)*, "protects the right to life, health, dignity and self-determination of the person and establishes that no health treatment can be initiated or continued if lacking the free and informed consent of the person concerned" (Cattorini et al. 2011). Every person who is capable of acting is recognized the right to refuse, in whole or in part, any diagnostic test or health treatment related to the disease from which the person is affected. The freedom of self-determination in the field of health care assumes such importance as to be recognized, in fact, even to those who are in a state of incapacity and it can also be exercised at a time prior to the onset of the disease or the situation that determines the state of incapacity through the *advance directive of treatment*. The ethical revolution of Biotestament has laid the foundation for the creation of a therapeutic alliance relationship between the one who provides care and the one who chooses to receive it, whereby the physician and/or the nurse can act according to the beneficial principle "only when the patient consents to his action and accepts it" (Garini et al. 2018). The recognition of the right to refuse treatment, however, is not equivalent to the recognition of the right to die, which is why it is important to know the meaning of the terminology that comes to the fore when discussing the end of life. The lack of training and information on

these aspects could generate confusion and reduced compliance; there are few studies conducted in the literature on these aspects.

2. Aim of the study

Survey of attitudes, perceptions and knowledge of the Italian population living in the provinces of Lecce and Brindisi with respect to the contents related to ethical issues of the end of life.

2. Methods

2.1 Design and Settings

The study was conducted among the population residing in the provinces of Lecce and Brindisi from December 2019 to February 2020. Before the start of the study, all participants were informed about the modalities and aims of the study. Only after their consent, the enrollment of study participants was carried out. The entire population in an age range of 18-80 years was included, who decided to take part in the survey on a voluntary basis. Each participant was given the data collection tool, the ad hoc constructed questionnaire consisting of a socio-demographic section and one relating to the actual survey on terms, content and attitudes related to the ethical issues of the end of life. To conduct this study, we chose to enlist a non-probabilistic sampling. The first items (1-12) inquire about the quality of the information related to the terminology concerning the ethical issues of the end of life, items 13-16 question and ask the respondent to express an opinion regarding the choice to suspend life treatments in cases of permanent vegetative state and regarding advance treatment dispositions related to adult and/or pediatric patients. Items 17-22 aim to explore in depth knowledge and attitudes about ART. Finally, the last two items (23-24) ask the respondent to express an opinion about the legalization of assisted suicide and euthanasia.

2.2. Statistical analysis

Analysis Descriptive analyses were conducted for all qualitative and quantitative variables using Statistical Package for Social Science (SPSS) Software version 17. Continuous variables were summarized by mean and standard deviation (SD) and categorical variables by frequencies and percentages.

2.3. Ethical consideration

Data were collected with respect for confidentiality and anonymity of the participants. Questionnaires were administered only to athletes who agreed to participate by signing the informed consent. The study project was illustrated and presented in advance to the facility managers of the centers involved; only after their consent was the survey started.

3. Results

3.1 Socio-demographic characteristics of the sample

The descriptive measures show that the population examined is evenly distributed between the provinces of Lecce (N=218, 54%) and Brindisi (N=186, 46%). The sample has an average age of 32 years (SD 13.14) More than half of the sample (n= 221, 54.7%) has a High School Diploma, the remainder (N=165, 40.8%) has a Degree. From the study, 52% (N=210) of the sample had never had the opportunity to train in bioethics, 46% (N=180) stated that they had done so through conferences and seminars, personal readings and through continuing education courses. (Table 1)

Table 1. Social-demographic data	**N (%)**
Age (average, DS)	32 (13.14)
Gender	
Male	156 (38,6)
Female	248 (61.4)
Civil Status	
Single	289 (71,5)
Married	89 (22.0)
Cohabitant	19 (4.7)
Divorced	7 (1.7)
Religious Creed	

Agnostic	56 (13,9)
Believer	254 (62,9)
Non-believer	94 (23,3)
Qualification	
No title	1 (0,2)
Elementary	3 (0,7)
Lower Middle	14 (3,5)
Superior	221 (54,7)
Under/post-graduate	165 (40,8)
Profession	
Physician	11 (2,7)
Nurse Practitioner	127 (31,4)
More	266 (65,8)
Children	
Yes	93 (23)
no	311 (77)
Have you had the opportunity to curate his education in bioethics before?	
No, never	210 (52)
Yes	180 (44,6)
Yes, through training courses	2 (0,5)
Yes, through conferences and seminars	6 (1,5)
Yes, through personal readings	6 (1,5)

3.1 Attitudes and knowledge with respect to living wills and end-of-life issues

Participants were asked to judge their own level of knowledge regarding ethical end-of-life issues. With respect to the regulatory aspect of three different clinical conditions such as "terminal illness" (47.8%), "irreversible coma" (36.6%) and "permanent vegetative state" (42.8%) it emerged that most of the participants have heard of them, so more than 40% in all three questions. 28.7% declared to know enough about terminal illness, while 40.1% declared to know enough about irreversible coma and 34.4% about permanent vegetative state. Despite what could be imagined, the analysis shows that the sample declares to feel quite knowledgeable in the field of Informed Consent (33.2%) in the same measure in which it affirms to have very good knowledge (33.2%), data that are subsequently confirmed since 57.2% are able to identify the correct expression of the meaning. For the Biological Will, as many as 85.8% of those interviewed would be able to define it and this could testify that the informed population is increasing, and *that Law 219/2017* has contributed to fill gaps on informed consent and to redefine the physician-patient relationship. Regarding the therapeutic field, which concerns both therapeutic abandonment and palliative care, the percentages show that the sample is well informed (44.5% and 35.6%). About palliative care, 28.5% of the sample felt they had very good knowledge. The majority of those interviewed chose the correct definitions, and the same situation was repeated for life-support treatments, which appear to be well known to almost all the sample, for which there were no doubts about the terms Nutrition and Artificial Ventilation (items 9-10). It is also noteworthy that, when asked to consider as fair the choice of a patient to refuse life-saving treatments, the sample agreed absolutely in 63.10% of cases, in line with the request to identify the most suitable figure to act in the place of the patient who cannot express his or her wishes: it is significant that 82.4% of the sample affirmed that therapeutic decisions are up to the patient who has signed the Living Will, while 5% believed that life-support treatments should never be suspended (item 13). It is also important that the participants believe that it is right that the will of minors in the clinical choice should no longer remain unheard, as shown by the percentages; in fact, there is a propensity on the part of the sample to listen to the opinion of the minor and to involve him/her in the choices that concern them. The figure that rises in the second case concerns the percentage of participants who consider the opinion of the minor to be irrelevant, which is almost 10%. One aspect that needs to be taken care of regarding advance treatment provisions is that of the way they are signed. The option chosen by 68.8% of the participants regarding the way to express the DAT is the written form authenticated by signature. Another part of the sample (16.1%) believes that the DAT can be communicated to a trusted person, while 12.6% (item 20) state that they do not know of any method. From an attitudinal point of view (item 21), the scenario is not homogeneous: 32.9% of the participants state that they feel sufficiently prepared, 31.4% that they have partial knowledge of the subject

and 23.3% that they do not feel sufficiently informed, together with 12.4% (N=50) of the sample who do not feel sufficiently informed at all. Regarding Euthanasia and Assisted Suicide, 50% of the population was in favor of the legalization of these two practices, respectively 50.7% for assisted suicide and 53.7% in favor of euthanasia. Those against are 5.4% for assisted suicide and 5.9% for euthanasia. The rest of the population is most likely to state that they are in favor with percentages of 32.9% and 29.7% and probably unfavorable for 10% (items 23-24) (Table 2).

Table 2. "Living will and end-of-life issues"	
1: Have you ever heard of irreversible conditions of illness with an inauspicious course, or physical/cerebral injuries where there is an inability to express one's own will and which force the patient to depend on people and machines?	**N (%)**
Yes, I heard about it on television.	160 (39,6)
Yes, through the internet.	128 (31,7)
Yes, through acquaintances.	101 (25)
No	8 (2)
Yes, through personal readings	6 (1,5)
Yes, through conferences and seminars	1 (0,1)
2.1 Terminal illnes Very poor I have heard about i I know enough Very good	19 (4,7) 193(47,8) 116 (28,7) 76 (18,8)
2.2 Irreversible coma Very poor I have heard about it I know enough Very good	 29 (7.2) 148 (36.6) 162 (40.1) 65 (16.1)
2.3 Permanent vegetative state Very poor I have heard about it I know enough Very good	 31 (7,7) 173 (42,8) 139 (34,4) 61 (15,1)
3: How do you rate your knowledge with respect to the following ethical issues and related standards governing the physician-patient relationship? (likert scale)	
3.1Informed consent Very poor I have heard about it I know enough Very good	 43 (10,6) 93 (23) 134 (33,2) 134 (33,2)
3.2Biological testament Very poor I have heard about it I know enough Very good	 46 (11,4) 144 (35,6) 154 (38,1) 60 (14.9)
3.3 Therapeutic abandonment Very poor I have heard about it I know enough Very good	 38 (9,4) 104 (25,7) 181 (44,8) 81 (20)
3.4 Palliative care Very poor I have heard about it I know enough Very good	 56 (13,9) 89 (22) 144 (35,6) 115 (28,5)
3.5 Assisted Suicide Very poor I have heard about it I know enough Very good	 59 (14,6) 155 (38,4) 141 (34,9) 49 (12,1)
3.6 Euthanasia Very poor I have heard about it I know enough Very good	 26 (6,4) 137 (33,9) 171 (42,1) 70 (17,3)
4: When we talk about a living will, what document are we referring to?	
To a document in which a patient asks for an end to their suffering, as painlessly and quickly as possible, in the case of incurable diseases.	59 (14,6)
To a document in which a patient asks to prolong his or her life by extraordinary technological means, in the case of incurable diseases	0
To a document, produced by a person in a lucid state of mind, regarding the possible care or treatment to which he or she wishes to be subjected at the time he or she becomes unconscious or loses decision-making capacity	345 (85,8)
To a document in which a patient asks to intentionally procure their own death if their quality of life is irreversibly impaired.	0
5: What is meant by euthanasia?	
It is a medical approach that aims to treat a patient through experimental treatments.	1 (0,2)
It is a medical and administrative aid that enables a patient to commit suicide independently and voluntarily, through an act performed by the patient.	113 (28,2)

It is a medical intervention intended to intentionally bring about the death of a patient, at his or her request and without causing pain, when his or her quality of life is irreversibly impaired.	284 (70,1)
I don't know.	6 (1,5)
6: What is palliative care?	
They are a medical approach that aims to treat a patient through experimental treatments.	10 (2,4)
These are treatments aimed at hastening the death of a terminal patient.	16 (4)
These are treatments aimed at delaying the death of a terminal patient as much as possible.	40 (9.9)
They are an approach that improves the quality of life for a terminally ill person and their family through prevention and relief of suffering.	338 (83,7)
7: What is meant by therapeutic overkill?	
It is the administration of medical treatment without the consent of the patient.	27 (6,7)
It is the administration of medical treatments that may not significantly benefit the patient.	323 (80)
It is the administration of treatments that are extraordinary but can provide significant benefits to the patient.	26 (6,4)
I don't know.	28 (6,9)
8: What is meant by assisted suicide?	
It is death brought about by the discontinuation of a medical treatment that keeps a patient alive.	60 (14,9)
It is the implementation of extraordinary treatments that expose the patient to a high risk of death or aggravation of his or her suffering.	9 (2,2)
It is suicide accomplished in person by a patient who has decided to die, with medical and administrative support.	206 (50,9)
It is the act of procuring the death of a patient at his or her request and with the direct intervention of a third party.	129 (31,9)
9: What is meant by artificial nutrition?	
It is the administration of saline by venous administration.	13 (3,2)
It is the washing of the intestinal or gastric walls with a saline solution.	3 (0,7)
It is the administration of nutrients by vein or through gastric or intestinal probes.	388 (96)
It is the help to take in food through feeding.	0
10: What is meant by artificial ventilation?	
It is a health care treatment that replaces or supplements the activity of the respiratory muscles.	308 (76,5)
It is a health care treatment that allows for the administration of oxygen intravenously.	14 (3,5)
This is a health care treatment whereby a flexible plastic tube is inserted into the pleural space.	59 (14,6)
I don't know.	**0**
11: Would you know how to define irreversible coma?	
It is a state of unconsciousness that could be modified because of a painful stimulus.	282 (69.8)
It is a state of brain death with the cessation of all brain function but with the persistence of cardiac activity.	117 (28.9)
This is a state of bedside immobilization of a quadriplegic patient.	4 (1.0)
I don't know.	1 (0,2)
12: Would you know how to define informed consent?	
It is the physician's obligation to have the patient read the medical record to let him or her know his or her medical condition and possible treatments to which he or she may be subjected.	98 (24,3)
It is the physician's obligation to inform the patient clearly about his or her medical condition and the possible treatments to which he or she may be subjected.	232 (57,4)
It is the physician's obligation to make a patient's family members aware of the medical condition and treatment they may be undergoing.	34 (8,5)
I don't know.	40 (9,9)
13: In your opinion, if a person has a brain disease or injury that prevents him or her from expressing their wishes and forces them to depend on machines, who should be responsible for any decision not to administer or suspend life-sustaining treatment?	
To the patient who has expressed his or her wishes through a living will, when it is available.	333 (82,4)
To immediate family members.	33 (8,2)
To the physician and health care provider treating him or her.	7 (1,7)
To an ethics committee.	4 (1)
To a legal guardian.	3 (0,7)
To a judge or magistrate.	4 (1)
Life support treatments should never be suspended.	20 (5)
14: In your opinion, if a person has not made their Advance Treatment Statements and is no longer able to	

express their wishes, who should make the decision to stop or continue treatment?	
To immediate family members.	242 (59,9)
To the physician and health care provider treating him or her.	46 (11,3)
To an ethics committee.	54 (13,4)
To a legal guardian.	32 (7,9)
To a judge or magistrate.	10 (2,5)
Treatment should never be suspended.	20 (5)
15: In your opinion, in the case of a minor patient over the age of 14, how involved should they be in decisions regarding their end-of-life in the event of a terminal or permanently disabling illness?	
Very much, the minor must be put in the condition to understand what his health condition and the possible developments of the disease and his will must be considered.	264 (65,3)
Partially, the minor must be put in the condition to understand what his health condition and the possible development of the disease is and to express his opinion, but the decision is up to others (family members, legal guardians, doctor).	112 (27,7)
Not at all, the child does not need to be informed and the decision rests solely with others.	28 (6,9)
16: In your opinion, in the case of a minor patient under the age of 14, how involved should they be in decisions regarding their end-of-life in the event of a terminal or permanently disabling illness?	
Very much, the minor must be put in the condition to understand what his health condition and the possible developments of the disease and his will must be considered.	217 (53,7)
Partially, the child must be put in a position to understand what his or her health condition is and the possible developments of the disease and to express his or her opinion, but the decision is up to others.	148 (36,6)
Not at all, the child does not have to be informed and the decision is solely up to others (family members, legal guardians, doctor).	39 (9,7)
17: The approval of the law on living wills introduces the Advanced Treatment Arrangements, which allow the patient to decide which treatments to undergo and their possible interruption. Are you in favor of this possibility?	
Absolutely.	289 (71,5)
Probably so.	102 (25.2)
Probably not	6 (1,5)
Absolutely not.	7 (1,7)
18: Do you think it is fair that a patient can choose, after being fully informed about the course of his or her illness, to refuse artificial nutrition and hydration?	
Absolutely.	255 (63,1)
Probably so.	105 (26)
Probably not	31 (7,7)
Absolutely not.	13 (3,2)
19: In your opinion, should Advance Treatment Arrangements be binding on the health care providers treating the patient?	
Absolutely.	205 (50,7)
Probably so.	145 (35,9)
Probably not	36 (8,9)
Absolutely not.	18 (4,5)
Item20: What ways are provided by law to express Advance Treatment Arrangements?	
They can be communicated to a trusted person.	65 (16,1)
They may be communicated in writing by authenticating the text.	278 (68,8)
They can be reported to the primary care physician.	7 (1,7)
They can be communicated through a video recording.	2 (0,5)
I have partial knowledge about	1 (0,2)
I don't know.	51 (12,6)
21: Do you feel prepared enough to write your own advance directive?	
Yes, I feel quite prepared.	133 (32,9)
I possess partial knowledge on this subject.	127 (31,4)
I am not sufficiently informed.	94 (23,3)
No, not at all.	50 (12,4)
22: How would you like to be documented about Advance Treatment Arrangements?	
I prefer to document myself (through the internet or books).	135 (33,4)
Through themed television programs.	44 (10,9)
Through flyers or newspapers.	10 (2,5)
Through your primary care physician.	54 (13,4)
Through conferences with experts.	154 (38,1)
Through university lectures.	6 (1,5)
I prefer not to document.	1 (0,2)
23: Do you support the legalization of assisted suicide?	
Absolutely.	205 (50,7)
Probably so.	133 (32,9)
Probably not	44 (10,9)

Absolutely not.	22 (5,4)
24: Do you support the legalization of euthanasia?	
Absolutely.	217 (53,7)
Probably so.	120 (29,7)
Probably not	43 (10,6)
Absolutely not.	24 (5,9)

4. Discussion

The objective of the study is to detect the qualitative degree of knowledge of the population under consideration with respect to the terminology and content related to the ethical issues of the end of life. To analyze the contents of this study, it is necessary to examine the constitutional reference point that is in Articles 2, 13 and 32 of our Constitutional Charter (Colacino 2015). The Italian Constitution has margins of pliability that allow it to be current even at time from its initial writing. The reference to art. 2 for the issue of health treatments consists in the affirmation of the existence of fundamental rights. We are always looking for a balance between the needs of the individual and the community, even if there are several factors that can alter the balance. The first knot to unravel is the misinformation on end-of-life issues as it emerges from the study by Lippo et al. (Lippo 2014) where about half of the respondents report not having enough information on some aspects such as irreversible coma and permanent vegetative state. Our study shows that more than half of the sample (52.0%, N=210) has never had the opportunity to educate themselves in the field of bioethics and almost the entire sample has been educated through the mass media or through the internet.

The task of the law is to try to provide a balance between the different opinions and compared to the past, the current era enhances this trend. A contribution in this sense has been made by jurisprudence, thanks to the entry into force of (*Law no. 219/2017*) (Rules on informed consent and advance treatment provisions) to which a not indifferent media explosion has followed, demonstrating how it has entered, before in our system, in the collective culture, drop by drop, through a series of cases widely documented in the press. The results of our study, show that 82.4% (N=333) of the sample, claim the decision to refuse, in the presence of certain conditions, a treatment potentially able to artificially extend his life, in contrast to what emerged from the study conducted by ISPO 2009 (Lippo 2014) in which only 50% of respondents recognized the right to self-determination of the subject. Another important aspect is certainly the end of life regarding the minor of age or incapacitated. *Law no. 4/2006*, instituted the figure of the support administrator, erasing the idea that the incapacitated person, if he reached a certain threshold of inability, should be deprived of any faculty of self-determination, replacing it with the principle of enhancement of every, even if minimal, possibility of autonomous exercise of the rights and faculties that pertain to him (De Filippis et al. 2018). Legislative and jurisprudential developments have enhanced the minor's capacity for discernment. From our study emerges a clear position of the sample to listen to the opinion of the minor and to involve him/her in the choices due to him/her. However, in the case of minors aged between 14-18 years, the percentage is 65.3%, which drops to 53.7% in the case of children under 14 years of age; this trend is reversed regarding the percentage of participants who consider the opinion of minors under 14 years of age as irrelevant, which is almost 10% higher. What emerges from the Biotestament law, obstinacy (the rule does not use the term overkill), which the physician must refrain from practicing, is configured in the case of an inauspicious short-term prognosis or imminence of death (Choi et al., 2015). In the public opinion, therapeutic overkill is identified in the administration of care that has no logical reason pe be practiced or continued, accompanied by suffering and / or risks to the patient, unnecessary or disproportionate. In this regard, the sample is well informed (80.4%, N=325). In any case, it is not insignificant that about a quarter of participants (N=104) still have only heard about

therapeutic obstinacy. It also does not go unnoticed that 5% (N=20) believe that life-support treatments should never be suspended, a fact that is not in line with what emerged from the ISPO Survey (Lippo 2014), namely that 20% of the sample believes that the decision to suspend should not be made by anyone. Paragraph 5, art. 1 (*Law no. 219/2017*), has central importance in defining the discipline of the law, puts an end to the age-old question of whether artificial nutrition and hydration are health treatments or should be otherwise defined. The prescription, in order not to be pleonastic, must be understood as an additional obligation, valid also for the case in which the information has already been provided. Physicians and/or nurses must inform patients of the consequences of such a decision and of possible alternatives, as well as promote supportive actions, also making use of psychological assistance services. In the context of these "life-sustaining" therapies, it was found that almost 100% of the sample responded correctly about artificial nutrition, while ideas appear less clear for artificial ventilation, where a small percentage of participants (15%, N=73) compared to the total sample provided an incorrect definition. Regarding Advanced Treatment Arrangements, 71.5% were fully in favor of their introduction, and 50% of the sample felt that the DAT were absolutely binding. This confirms that the population is increasingly concerned about the right to self-determination in the field of healthcare. The option chosen by most participants regarding the method of expressing DAT is the written form authenticated by signature. Not to be overlooked is the fact that 12.6% of the participants declare that they do not know of any method. The respondents were asked once again about their aptitude for writing their own Anticipated Treatment Arrangements: 32.9% (N=133) of them stated that they felt well prepared, 31.4% (N=127) that they had partial knowledge of the subject, and 23.3% (N=94) that they did not feel sufficiently well-informed, together with 12.4% (N=50) of the sample who did not feel well-informed at all. A large part of the sample affirms that it would like to broaden its knowledge about DAT through conferences with experts or by seeking information on its own and from general practitioners. The DAT (Declaration of Anticipated Treatment) can be drawn up by the citizen without any support, even if, as for the will, the collaboration of a lawyer may be opportune, as well as the help of a physician, a nurse, to ensure that they are clear and comprehensible and do not give rise to problems of interpretation. In the absence of a DAT, the norm regulates the conflict between the representative of the incapacitated person and the physician, should the former refuse the treatment proposed by the latter. While in the case of a capable subject or DAT, the physician is obliged to respect the expressed will, as established by paragraph 6 of art. 1. As far as assisted suicide is concerned, the sample is almost evenly distributed between those who claim to be well-informed (34.9%, N=141) and those who have only heard of it (38.4% N=155), and those who, to the extent of 14.6% (N=59) have very little knowledge about it. In any case, 50% (N=206) of the respondents were able to give the correct answer. With respect to the condition linked to euthanasia, 42.1% (N=170) of the sample believe they are well informed. Even in this case, however, it should be underlined that 33.9% (N=137) have only heard about it. Regarding the possibility of legalizing assisted suicide and euthanasia, more than 50% of the sample were fully in favor of the legalization of these two practices. It should be emphasized that euthanasia and biotesting differ in the same way that action differs from omission, as well as differing by having the one aim to oppose nature, the other to indulge it. Closer to active euthanasia is assisted suicide, which consists of life-ending activities carried out directly by the person concerned, with the assistance or help of a third party. Health care professionals have a key role in clarifying some aspects of this new legislative framework for citizens. They work with people in a high state of dependency and need, in a situation made even more difficult by the presence of numerous hierarchies and cultures. Nurses have a

responsibility towards institutions and citizens, finding themselves in the ambiguous, but unique and potentially rich position of arriving at a collaborative choice. It should be noted that, due to the small size of the sample (although not negligible) and the minimal territorial extension, the results cannot be generalized at the national level.

5. Conclusions

The objective of the study was to detect the knowledge, perceptions and attitudes of the Italian population living in the provinces of Lecce and Brindisi with respect to the contents related to the ethical issues of the end of life. The results of the study showed that knowledge on the subject has definitely improved over the years and that in most of the issues addressed, the population has an adequate degree of preparation even if there is still some skepticism in addressing issues such as assisted suicide and euthanasia. Regarding the perception of knowledge about certain clinical conditions at the end of life (terminal illness, irreversible coma, and permanent vegetative state), it was found that most of the participants are aware of these situations thanks to the news conveyed to the public by the mass media such as TV and Internet, demonstrating that there is a greater awareness of the issue of the end of life. The deficient aspect to be taken care of in the field of advance treatment provisions is the one concerning the modalities of subscription; considering that the sample under examination declares not to know any modality and that one person out of 404 would prefer not to inquire, it becomes a challenge to identify strategies for the dissemination of information that can be used by the widest possible portion of the population. About euthanasia, it should be emphasized that a large part of the sample has only heard of it, confirming the fact that in Italy we are still far from considering it an acceptable practice, even though there is a large percentage, equivalent to more than half of the sample, in favor of its legalization. The situation is identical regarding the legalization of assisted suicide. The End of Life is part of the path of every individual and for the same reason it is right that it should become the object of conscious choices. A reasoned and shared approach would be necessary to clarify the accuracy of the terms to make "meditated and conscious individual choices in light of the principle of Self-determination (autonomy) (item 4)". It is necessary to promote information campaigns that can guide the population to the correct approach to the End of Life through the introduction of training tools and effective dissemination strategies for the correct drafting of one's DAT, to shed light on realities that, in the light of medical and scientific progress, prove to be of crucial importance and allow the patient to expand his or her *HABEAS CORPUS* now more than ever.

6. References

- Beauchamp, TL., Childress, JF 2001. "Principles of Biomedical Ethics". *Oxford University Press, USA.*
- Carlet, J., Thijs, LG., Antonelli, M., Cassell, J., Cox, P., Hill, N., Hinds, C., Pimentel, J. M., Reinhart, K., and Thompson, BT. 2004. "Challenges in end-of-life care in the ICU". *Intensive Care Medicine*, 30(5), 770-784.
- Cattorini, PM. 2015. "Bioetica. Metodo ed elementi di base per affrontare problemi clinici". Elsevier.
- Choi, Y., Keam, B., Kim, TM., Lee, SH., Kim, DW., and Heo, DS. 2015. "Cancer Treatment near the End-of-Life Becomes More Aggressive: Changes in Trend during 10 Years at a Single Institute. *Cancer Research and Treatment: official Journal of Korean Cancer Association,* 47(4), 555.
- Colacino, G. 2015. " Autonomia Privata e Direttive Anticipate". A. Giuffrè Editor, Milan, Italy.
- De Filippis, B. (2018). "Biotestamento e Fine Vita". CEDAM.
- Garini, G. 2018. "ALCUNE RIFLESSIONI SU PRINCIPIO DI AUTONOMIA, CONSENSO INFORMATO E DISPOSIZIONI ANTICIPATE DI

TRATTAMENTO". *I mercoledì della Bioetica*. https://www.normattiva.it/urires/N2Ls?urn:nir:stato:legge:2017;219.

- Lippo, L., Zenobi, C., and Amato, MG. 2012. "Il Testamento Biologico: Una scelta consapevole? Sondaggio sulle conoscenze degli italiani". *PROFESSIONI INFERMIERISTICHE*, 65(3), 171–178.
- Riccioni, L., Busca, MT., Busatta, L., Orsi, L., and Gristina, GR. 2016. "La limitazione dei trattamenti: Una forma di eutanasia? Un approccio scientifico al dibattito sulle decisioni alla fine della vita." *Recenti Progressi in Medicina,* 107(3), 128.

JDReAM

http://siba-ese.unisalento.it/index.php/jdream

Lecce