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Voice Biometrics Technologies and Applications for Healthcare: an overview.

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Abstract
Our medical information is perhaps considered the most personal and valuable of all. On the other hand, the rapid increase in the digitization of the healthcare industry nowadays is giving the patient identification and electronic medical record maintenance a key role to improve patient safety, protect against medical identity theft, enhance patient satisfaction, not to mention financial performances. Regulatory pressures and compliance guidelines naturally require health care providers to handle patient information with the highest degree of privacy and care. In this scenario, innovative healthcare organizations are increasingly using biometric technologies to support smoother and safer patient access and information sharing to improve security. Biometric identifiers are measurable unique characteristics of an individual physiological (such as fingerprints, iris, retina, DNA) or behavioural (such as typing rhythm, voice, and gait) traits that can be used to positively identify a person. Among them, voice biometrics is actually a convenient and secure method of authenticating a speaker's identity using just a few words, which has many advantages over other forms of biometric identification especially in the healthcare sector. After briefly discussing the impact of biometric technologies from a general point of view, the present paper focuses on voice biometrics, to give a technical understanding of how it works according to the current state of art and the forthcoming progress, and how it can be successfully integrated in the healthcare systems.

Keywords: voice biometrics, speaker recognition, patients identification, staff authentication.

Introduction

Among the many challenges facing healthcare today is the need of costs containment while improving the quality of care. To overcome these challenges, it seems increasingly necessary to efficiently ensure the proper identification of all the healthcare constituencies (staff, providers, payers and patients). The failure to correctly identify patients opens the doors to frauds and, even more seriously, to patient identity (ID) theft. The identity security is even more important in the healthcare sector than in others, because other than costly medical errors, identity theft can prevent legitimate people to access critical medical services, with consequences that can become dramatic. Moreover, unlike other sectors such as financial, frauds can easily go undetected for a long time. Healthcare frauds can be done, for example, by providing false information when applying for programs or services, forging prescriptions or selling prescription drugs. Also, medical records have a very high transactional value in black markets compared, for instance, to stolen credit information, because of the huge amount of data about a particular victim that is available through these records. Moreover, patient records are sensitive and their safety is a regulatory requirement. Although statistics do not always agree about the costs of healthcare frauds, some authoritative estimates ranged around 6.19% of global healthcare expenditure, equivalent to €350 billion (PKF Lillteljohn LLP and University of Portsmouth, 2015).

The most important reason why such kind of attacks have been able to occur is the vulnerability of account protection and authentication methods, and this is why the healthcare industry is being increasingly attracted by the biometric technologies. This is confirmed also by the most recent market researches, that estimate that the global healthcare biometrics market will grow at an impressive CAGR of 22.9% to surpass $13.9 million in revenue in 2025

(http://www.credenceresearch.com/report/healthcare-biometrics-market), with North America and Europe together capturing over 70% of the global healthcare biometrics market.

Biometrics in the healthcare industry can be used for applications in doctors' offices, hospitals, or for use in monitoring patients, to implement logical access control to centralized archives of digitized patients' data, physical access control to buildings and hospital wards, workforce management, patient record storage, authenticate medical and social support personnel. Voice biometrics is a mature enough technology to provide the required levels of security, versatility, cost effectiveness, preference by users, which only are required to speak to get automatically recognized.

This paper is organized as follows. Section 2 introduces some essentials about biometric recognition principles and definitions. Sections 3 and 4 gives a more technical overview of the voice biometrics concepts and the techniques used up to the current state of the art. In Section 5 the main advantages of voice biometrics over other kinds of biometric recognition in healthcare are discussed. Finally, conclusions are presented in Section 6.

Biometrics: some fundamentals.

The terms "Biometrics" and "Biometry", from the Greek root of βίος (bíos, i.e. "life") and μέτρο (mètron, i.e. "measure"), literally mean the "measurement of life", and refer to a methodological process that confirms its classification as a science. Biometrics, therefore, is a systematization of applicable knowledge to experimentally controllable hypotheses (Preite, 2016). Since the studies started in 1890 by Francis Galton (1822-1911) and later by Karl Pearson (1857-1936), the terms "Biometrics" and "Biometry", have been used traditionally in reference to mathematical methods to address data analysis problems in the biological sciences, indeed they are also referred to as "biological statistics" or "biostatistics".

On the other hand, recently, the term "Biometrics" is increasingly used to refer to the (auto-matic) identification and authentication of an individual by means of one or more measurable unique traits (Figure 1). Such biometric identifiers can be divided in two categories: "Anatomic-Physiological", and "Behavioural". The first category includes face traits and thermography, fingerprints, hand geometry, palm veins, iris, retina and DNA recognition, while typing rhythm, voice and gait come under behavioural biometrics.

With respect to traditional approaches that identify a person on the basis of a key or a token, physical (something an individual has) or intangible (something an individual knows, e.g. a password, a Personal Identification Number, PIN), in the biometric approach the individual itself becomes the key (something an individual is). Moreover, biometrics can be used in combination with those traditional approaches, leading to three-factors security level (Figure 2). Generally, biometric schemes fall into two categories: "Authentication" and "Identification". In the authentication, the individual makes a claim of identity and the biometric recognizer has to verify if that claim is true or not, comparing the input features with those of the claimed identity (which was stored within an early enrollment stage). Authentication is therefore also referred to as "Verification", and has a binary output: accepted or rejected. In the identification approach there is no identity claim and the recognizer has to compare the input biometric features of the unknown individual against those of each individual in a set of pre-enrolled ones, producing a score for each comparison, and returning the identity of the enrolled person corresponding to the best score. If the system is allowed to reject the identification request when the highest score is still very low compared to a certain threshold, the identification is named "open-set"; on the contrary, if the system must return in any case the best matching identity, the identification is named "closed-set".
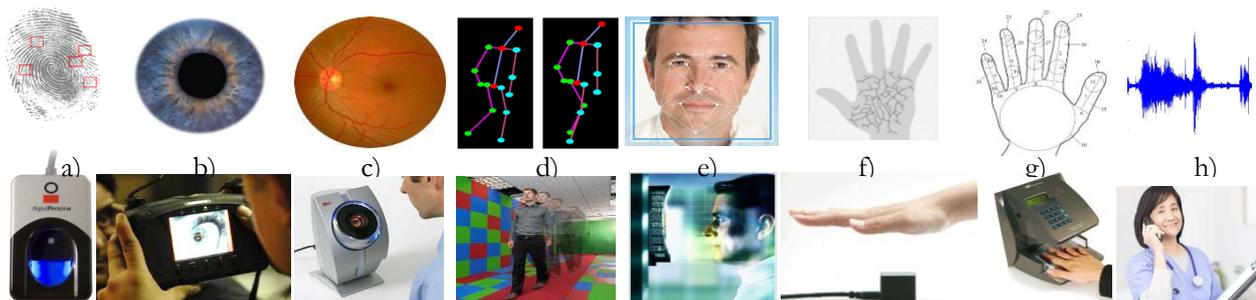
Figure 1. Some biometric identifiers and related devices. a) Fingerprint recognition looks for the unique patterns of ridges and valleys that are present in an individual's fingerprint, that is acquired by a specific hardware scanner, often in combination of temperature and pulse to improve the authority of the image; b) Iris recognition methodologies rely on image processing techniques to identify patterns in iris images, that need to be acquired by a scanning device; c) Retina recognition relies on the property of the blood vessels to absorb light more readily than the surrounding tissue; a retinal scanner is able to produce the appropriate lighting and acquiring the resulting pattern; d) Gait biometrics identifies and recognises people by the way they walk; individuals may be required to provide "gait" samples by walking through a tunnel where sensors are able to capture the relevant features; e) Face recognition algorithms typically identify facial features by extracting landmarks, or features, from an image of the subject's face; f) Palm vein recognition is generally based on the capability of deoxidized haemoglobin flowing through the veins to absorb near-infrared light, provided by a palm scanner., making them appear as dark pattern; g) Hand geometry recognition is based on measuring and recording the length, width, thickness, and surface area of an individual's hand while guided on a plate, by means of a specific device; h) voice biometrics is discussed more in detail in the present work.
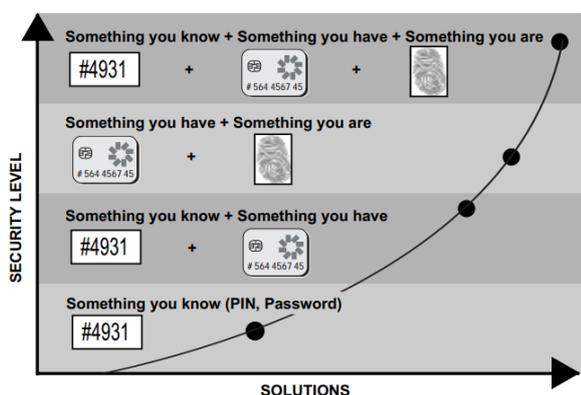
Figure 2: combining biometrics with traditional approaches improves the security level of authentication (Smart Card Alliance 2002).

As one can already guess by the above statements, biometric approaches are "Probabilistic", in the sense that the outcome is always intrinsically provided together with an estimate of the decision error. On the contrary, identification approaches such as the password-based one, in which the system can provide a sure match of all of the characters and case of the password, are named "Authoritative".

Performances of biometric approaches can therefore be measured on the basis of the extent of the errors they make while providing the outcome. Considering for instance a biometric authentication system, two kind of errors can be done: a "False Accept" (or "Miss"), when the system authenticates an impostor, and a "False Reject" when the system refuse a true identity claim. Accordingly, the system is designed to achieve an average "False Reject Rate" (FRR, also named type I error) and an average "False Accept Rate" (FAR, also named type II error), as a result of a trade-off between the two error types. Many other metrics can be defined on the basis of FAR and FRR, such as the "Equal Error Rate" (EER), which is the defined as the operating point where FAR equals the FRR, corresponding to an optimally chosen value of some parameter, which is typically a threshold for a some kind of similarity scoring function (see Figure 3).

Figure 3: The score distributions for non-target (left) and target (right) trials. The grey areas left and right of the threshold represent P_{MISS} (which is equivalent to FRR) and P_{FA} (equivalent to FAR) respectively (van Leeuwen and Brümmer 2007).

On the other hand, the optimal operating point can also be chosen considering unequal costs for FA and FR errors (e.g. when higher FRR can be tolerated with respect to FAR), trying to minimize the following Detection Cost Function (DCF) introduced by the NIST (the well-known American National Institute of Standards and Technologies) over years:

$$DCF(\tau) = C_{MISS}P_{MISS}(\tau)P_{Target} + C_{FA}P_{FA}(\tau)(1$$

where P_{Target} is the prior probability of a specific target speaker, C_{MISS} and C_{FA} are fixed costs, that depend on the specific application, for the False Reject (i.e. Miss) and False Accept events, and P_{MISS} and P_{FA} are the probability of False Reject and False Accept events both as function of the score threshold τ. Both FAR and FRR depends dramatically on the chosen biometric identifiers and on the actual features that can be extracted from them. Provided that, in general, in biometric applications, no feature can assume always the same value over time, or, at least, can be extracted without some kind of superimposed noise, the key point is that the best features are those that, at the input of the biometric system, exhibit the smaller variability within the same individual, the greater variability across different individuals and the best r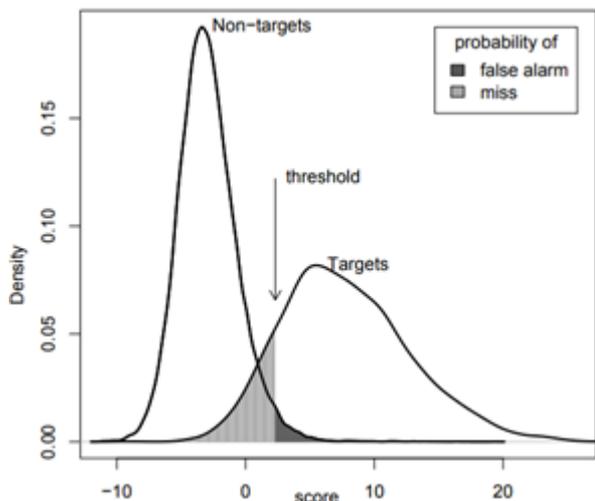obustness against noise. This is extremely important mainly for biometric identifiers such as the human voice, which is intrinsically charac-

terized by a high degree of variability and, due to its versatility, can be used in many different scenarios and use cases in different environmental conditions.

Speaker recognition: the voice as biometric identifier.
Many dictionaries and textbooks use the term "Voice" and "Speech" interchangeably. However, from a scientific perspective, the two terms have different meanings. Voice (or vocalization) is the sound produced by the vibration of the vocal folds, in the larynx (or voice box), as the result of the pressure of the airflow produced by the lungs. Speech is an information-rich signal produced by a series of complex movements that, by means of frequency, amplitude and time modulation, change the basic tone created by voice into specific, decodable sounds (phonemes), that actually can be voiced or unvoiced (i.e. with or without vibration of the vocal cords), to convey information about words, identity, accent, expression, speaking style, emotion and the state of the speaker[1].
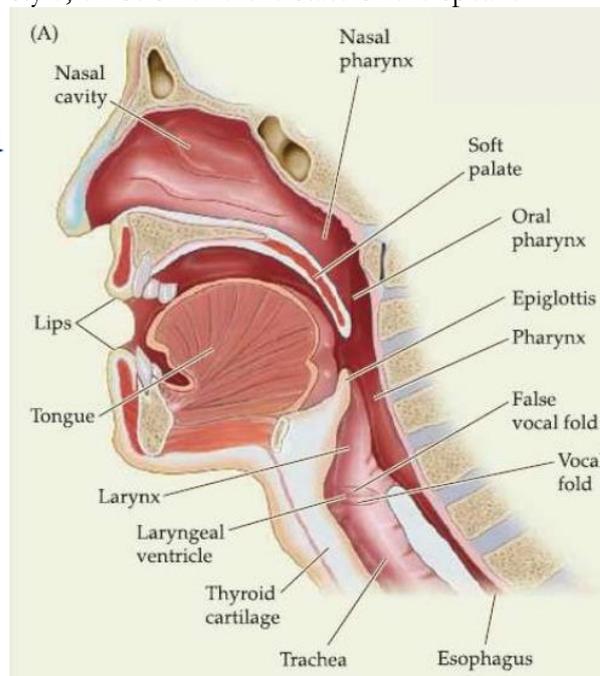
Figure 4: The peripheral phonation system (Purves, 2012).

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[1] For sake of completeness, we report also a definition of "Language", as "the expression of human communication through which knowledge, belief, and behaviour can be experienced, explained, and shared. This sharing is based on systematic, conventionally used signs, sounds, gestures, or marks that convey understood meanings within a group or community" (National Institute on Deafness and Other Communication Disorders, https://www.nidcd.nih.gov/).

The distinction between Voice and Speech is also relevant to distinguish "Voice recognition" from "Speech recognition" applications. Voice recognition, Speaker recognition (or "Identification", "Authentication", "Verification") are all about the recognition of the speaker identity, i.e. the aim of Voice biometrics, while "Speech recognition" is largely used to address the task to recognize the spoken phonemes, words and sentences, i.e. the content of the speech. Strictly speaking, the recognition of what is said is not the field of voice biometrics, but as will be explained later in the present work, it can be successful combined with speaker recognition to increase the security of the voice-based recognition system, preventing impostors to be acknowledged thanks to fake pre-recorded utterances.

Speaker recognition is a complex operation that always start with the capture and processing of the speech signal, therefore, for this purpose, "Voice" and "Speech" can continue to be used interchangeably.

Speaker recognition is not a very recent concept, but, along with other biometric identification methodologies, it has become more accessible and affordable in recent times. Based on the pioneering studies started in 1960 by Gunnar Fant at the KTH Royal Institute of Technology (KTH, Swedish: Kungliga Tekniska högskolan) in Stockholm, Sweden, about the physiological components of acoustic speech production, and later expanded also by Joseph Perkell, in 1976 Texas Instruments built a first speaker recognition prototype system that was tested by the U.S. Air Force and the MITRE Corporation. Since then, a constant increasing interest has grown for the biometry of speech, and many progresses have been made in parallel with the development of technologies in the field of electronics, software engineering and digital signal processing and analysis.

The task of infer the identity of an individual by his/her voice is a challenging one, also because of the large intrinsic variability of the speech signal (other than the many factors depicted in Figure 5). However, this variability is associated with the ability to convey a huge variety and quantity of information, and it is what makes the voice the most natural form of human communication.
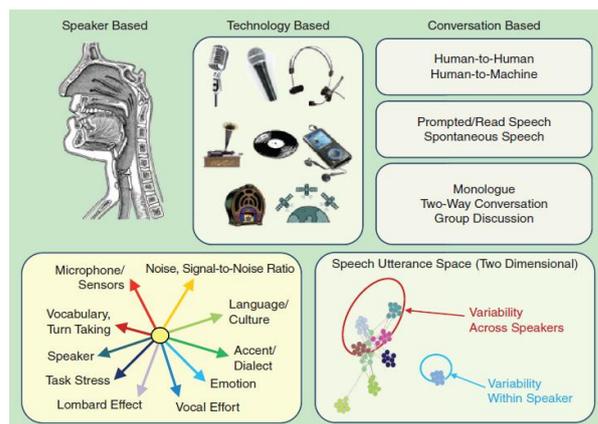
Figure 5: Source of variability in Speaker Recognition (Hansen and Hasan 2015).

In order the determine the identity of a speaker given one or more utterance, not the whole speech signal is considered, but only a set of measurements or "features". As the speech production is influenced by a given speaker in many different ways, features can be extracted at different levels as depicted in Figure 6. Other than maximizing the variability among different individuals and minimizing the variability within a given person, the ideal speaker features for biometric applications should also be difficult to mimic, be robust against the change in the speaker's health or long-term variations in voice, occur frequently and naturally in speech, be robust against noises and distortions.
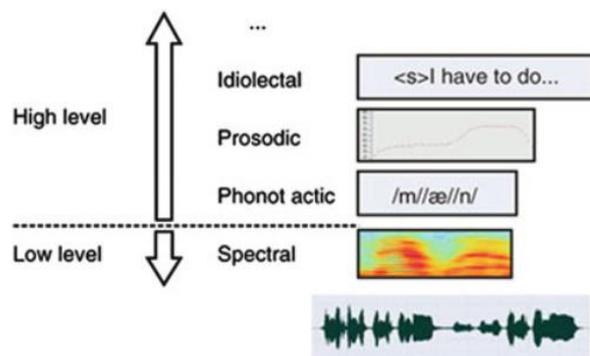
Figure 6: identity levels in the speech signal (Ramos et al. 2015).

Despite several studies shows the significance of suprasegmental (high level) features in speaker recognition systems (Demenko 2000, Yegnanarayana et al. 2001 and 2005), the low-level short-term spectral features are the most used, also because they reflect, more or less, the physical characteristics of the vocal tract. The concept of short-term spectral features can be easily depicted considering for example the Mel-Frequency Cepstral Coefficients (MFCCs,

Davis and Mermelstein, 1980), widely adopted features that have been proved to provide quite low error rates for clean signal conditions. Basically, the MFCCs are based on a linear cosine transform of a log power spectrum on a non-linear mel scale of frequency.

The whole utterance is divided in segments of duration of approximately 20-30 ms (hence the expression "short-term") with a certain amount of overlap (Figure 7). As depicted in Figure 8, for each frame, a power spectrum estimate is computed by means of Discrete Fourier Transform (DFT) and several other processing steps occur in order to achieve a bunch of numerical coefficients, which are the actual features. In the case of MFCCs, the power spectrum is input to a filter bank whose bands are equally spaced on the mel scale (see Figure 9), to better approximates the human auditory system's response.
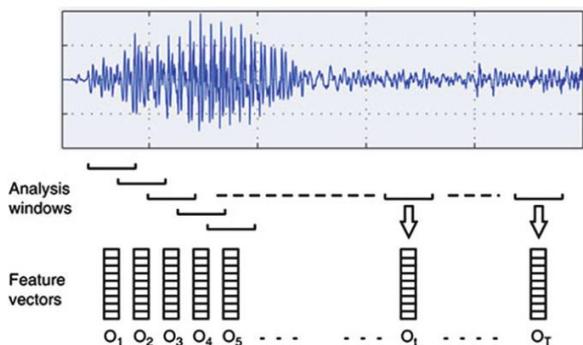
Figure 7: Overlapping framing of the acoustic waveform and frame-by-frame feature extractions. (Ramos et al. 2015).

Feature extraction outputs a temporal sequence of acoustic vectors $X = \{o_1, o_2, \dots o_N\}$ of length N with each vector o_n having a constant dimension D.

Many other "flavours" of short-term spectral features have been proposed in order to increase robustness against noise and other disturbance affecting the speech signal, such as the Gammatone Frequency Cepstral Coefficients, GFCCs (Chanwoo et al. 2012), the Power Normalized Cepstral Coefficients, PNCCs (Al-Kaltakchi et al. 2016), and many others. Motivated by the fact that the neural responses are robust against noise, a research group has also proposed "neural" features as extracted from the responses of a physiologically-based computational model of the peripheral level of the auditory system (Islam et al. 2016).

The robustness against noise and channel mismatch is one of the true challenge for speaker recognition systems, mainly for those applications where the quality of the captured speech signal cannot be guaranteed. Even the environmental noise cannot always guaranteed to be a stationary process, because of the large variability of noise sources (reverberations, mechanical noises, even undesired voices of other speakers – the "cocktail party" effect). The speaker features may also get affected by the so called "channel mismatch", when the same individual could present himself to the recognizer using many devices – PC, smartphone, etc.- and transmission channels – mobile or landline telephone, data connections with different encodings and capacity), depending on the use cases.
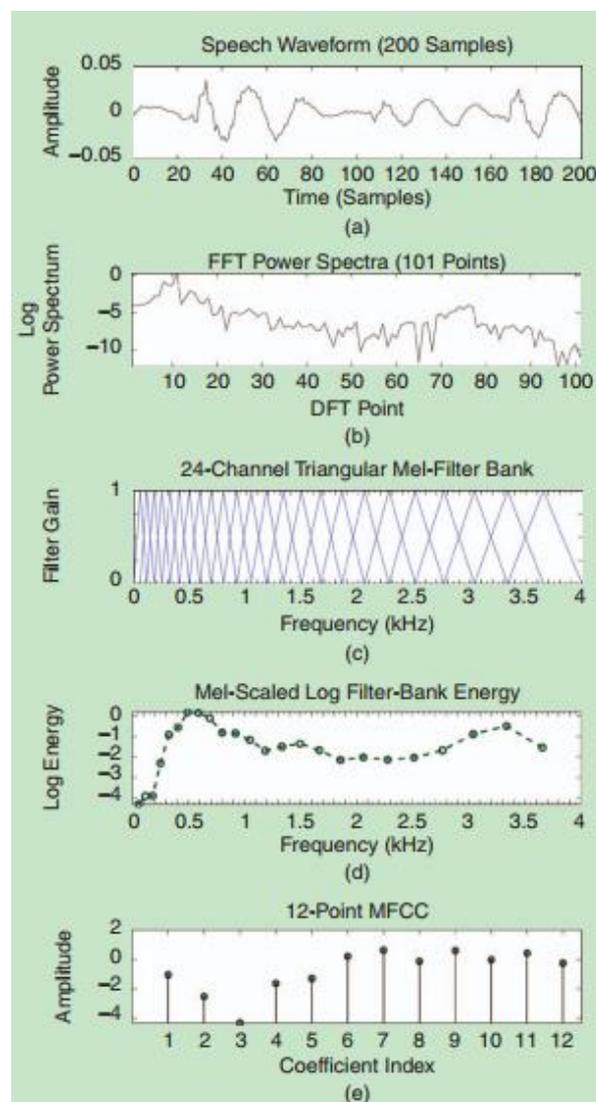
Figure 8: Steps in MFCCs feature extraction from a speech frame: (a) 200-sample frame representing 25 milliseconds of speech sampled at a rate of 8 kHz, (b) DFT power spectrum showing first 101 points, (c) 24-channel triangular Mel-filter bank, (d) log filter-bank energy outputs from Mel-filter, (e) 12

static MFCCs obtained by performing DCT on filter-bank energy coefficients and retaining the first 12 values (Hansen and Hasan 2015).
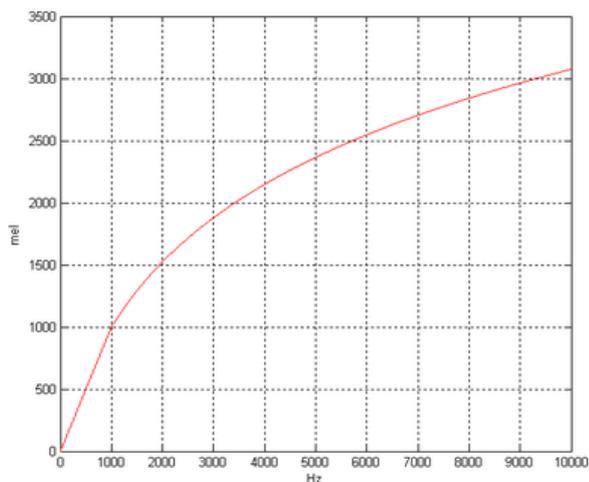
Figure 9: A plot of the mel transform. Horizontal axis: the frequency in Hz. Vertical axis: the corresponding mel values.

For examples, a doctor may need to be authenticated to access a reserved patient record from the desktop computer in the silence of his own office, or in a crowded loud department through a mobile handheld, or even through a simple voice call. In order to achieve robustness, i.e. low error rates, a huge amount of optimizations have been proposed by researchers all around the world, not only at the feature extraction level, but also throughout at architectural level and at each building block.

Anatomy and functioning of an Automatic Speaker Recognition system.

samples coming from many individuals, which represent the background population, bringing information about the variability of speech among different individuals (inter-speakers variability). In a next phase, the models of single authorized speakers can be trained, taking advantage of the background model computed in the first stage. Once the background and the authorized speaker models have been trained, the recognizer is ready to infer the identity of the incoming speakers (the "test" phase). In all phases, the voice samples must be captured and digitally converted, pre-processed (e.g. denoised), then entered in the feature extraction block, which typically produces a time series of features vectors. In each training phase, the features are processed to get a model of the speaker, which is stored in a database. In the test phase, the features of the test speaker are matched against the model(s) of the enrolled speaker(s) stored in the database for authentication (or identification).

So, after the feature extraction, a key role is played by the speaker modelling, i.e. the computation of a summary description of the feature distribution of a given speaker, and the related matching strategy. Many models have been proposed, such as the Vector Quantization technique, VQ (Burton 1987), where the feature vectors of the enrolled speakers are used to learn an optimal clustering (a codebook), specific to each speaker, of the feature
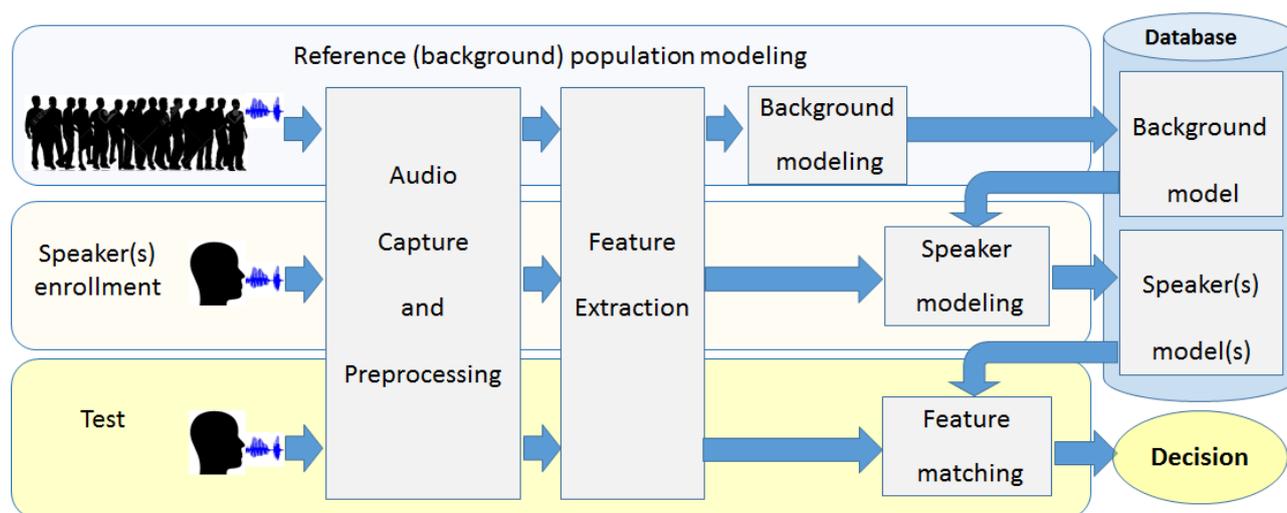
Figure 10: Schematics of a speaker recognition system.

Figure 10 shows a schematic of a typical speaker recognition system. In a first phase, the system needs to be trained with a number of voice

space. However, a first breakthrough came when the probabilistic approach was introduced, with the idea of modelling the variability

of each speaker with a probability density given by a mixture of multidimensional Gaussian components (GMM, Gaussian Mixture Model, Reynolds and Rose 1995), trained applying an Expectation Maximization (EM) algorithm.

In a basic identification task, the system returns the identity of the enrolled speaker whose GMM exhibits the highest statistical likelihood, given the feature vectors of the test speaker. In order to address the authentication task, a GMM is also trained with a large set of feature vectors coming from the utterances of many speakers, in order to get a so-called "Universal Background Model", UBM), for a reference population of speakers (Reynold et al. 2000). In this case, the feature vectors of the test speaker could be used to compute a likelihood ratio (LR) between the GMM of the claimed identity and the UBM, while the final response, Accept or Reject, could be provided comparing the LR to a threshold value.

A second breakthrough came with the introduction of the "supervectors", fixed-length vectors built by stacking the centroids of the GMMs components, that could be used in other processing steps, such as Support Vectors Machines, SVMs (Campbell et al. 2006). The latter is a machine learning classification technique that aims at optimally separating multidimensional data points (as the supervectors) from two classes (e.g. the enrolled speaker and the reference population) using a hyperplane, as depicted in Figure 11.

After the learning phase, if the supervector of the test speaker falls in the region associated to the claimed identity, the speaker is accepted.

Supervectors opened the doors to further improvements, because they made possible to apply Factor Analysis (FA), such as the Join Factor Analysis, JFA (Kenny 2005, Kenny et al. 2007) to separate (and discard) estimated contributes of noise and channel from that of the speaker, and, at the same time, to allow a some dimensionality reduction that make more practicable computational expensive tasks such as SVM training.
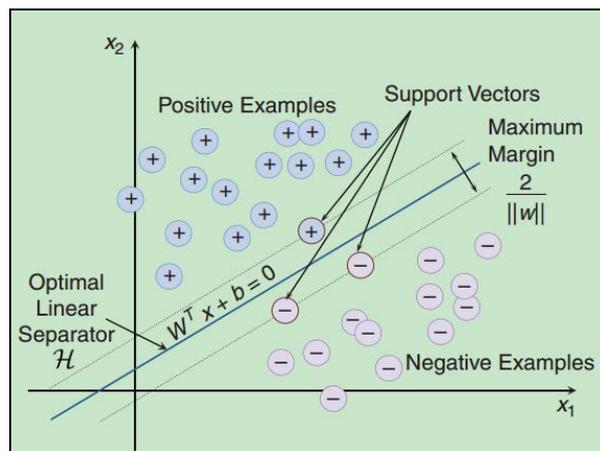
Figure 11: A conceptual illustration of a SVM classifier (Hansen and Hasan 2015). When applied to speaker identification, positive (+) points represent the vectors of utterances of the claimed identity, while negative (-) points represent utterances of the speakers belonging to the background population.

Moving from JFA, which was the yesterday state-of-art, some researchers introduced a new low-dimensional speaker- and channel-dependent space, defined using a simple factor analysis. The so called Total variability Space modelling (TVS, also known as "i-vectors" approach, cfr. Dehak et al. 2009 and 2011) provided a way to more effectively summarize the speakers utterances allowing at the same time a further dimensionality reduction and the use of noise and channel compensation methods that were not practical in large dimensional supervectors, such as Linear Discriminant Analysis, LDA (Vogt et al. 2008), Nuisance Attribute Projection, NAP (Campbell et al. 2006b), Within-Class Covariance Normalization, WCCN (Hatch et al. 2006). In the current state-of-start, the GMM-UBM/i-vector approach is combined with Probabilistic Linear Discriminant Analysis, PLDA (see Kenny 2011, Garcia-Romero and Espy-Wilson, 2011), a technique first used for session variability compensation for facial recognition, which works in principle as factor analysis in the i-vectors space (instead of the supervectors space). This allows an even further effective dimensionality reduction, which results in a more effective training phase and a simpler implementation. In the GMM-UBM/i-vectors/PLDA framework, the recognition is finally achieved comparing the LR of two alternative hypothesis, i.e. that the speaker-dependent component of the i-vectors of the test speaker and the claimed identity belong to the same identity, or not, with a threshold value.

The current state of art is rapidly moving toward Artificial Neural Networks (ANN), in replacement or in addition to some module in the traditional architecture. Deep Neural Networks (DNNs), large feed-forward ANNs with several (roughly 5-7) hidden layers and thousands of nodes, have recently been successfully used for acoustic modelling for the Automatic Speech Recognition task, achieving large improvements compared to standard GMM models. Researchers in the field of speaker recognition are proposing to use DNNs, and other kinds of ANNs such as the Convolutional Neural Networks (CNNs, see McLaren et al. 2015), as replacement of the UBM module (leading to a DNN/i-vector framework; see Lei et al. 2014), as de-noising module, as feature extractor module, leading to EER values up to 1-2% mainly in clean conditions, with an improvement over the traditional approach up to 30%. Although in the field of voice biometrics there is still a lot of room for improvements, as for other biometrics technologies, the current state-of-art is ready to be successfully used in many sectors, including healthcare.

Voice biometrics for healthcare.
As already mentioned, biometric identifiers can be anything from fingerprints, palm veins, and iris scans to gait, facial, other than speaker recognition. Each one has its own benefits and disadvantages, but the latter has unique features that make it ideal for the healthcare sector, such as:

- Familiarity: using their own voice to interact with devices is becoming more and more familiar as voice-activated personal assistants and mobile apps spread.
- Little hardware required and cost effectiveness: any device equipped with a microphone and a network connection (voice or data) is ready. Voice biometrics user interfaces require no expensive scanning devices, only existing phone systems and everyday technology.
- No training is required to use a voice biometric system, because speaking is a natural activity.
- Mobility: voice biometrics works with any telephony technology, from anywhere in the world. Moreover, it is the only practical biometric option for authentication/identification over telephone channel.
- Accuracy: improvements in speech digital signal processing, such as noise cancelling and voice activity detection, as well as machine learning and deep learning are shrinking error rate under 1% in the most operative conditions.
- Effectiveness: as voice biometrics is actually a combination of both physical attributes (the shape of one's vocal tract) and behavioural attributes (speaking style), with a single interaction it provides authentication as well as "liveness testing." When used with speech recognition, voice biometrics can also be used to gather additional information such as a passwords, PIN, user-acknowledgement of prompted questions and so on, if required, with little or no extra cost.
- Integration with existing security systems: voice biometrics can be used with existing authentication methods to provide high-security multi-factor user-authentication.

Thanks to these concurrent properties, voice biometrics is an optimal solution, both as a stand-alone strategy and combined with traditional security approaches or other biometric identifiers for critical security needs, to improve the quality of healthcare services reducing costs at the same time.

Not only doctors but in general the healthcare staff as well as patients can get used easily with voice authentication and identification because recognition can happen in a seamless way, as if they speak at their own voice-activated personal app.

Electronic patient records can be safely retrieved by authorized personnel only, everywhere in the medical facility, by means of ordinary tablets and smartphones, other than desktop personal computers.

Simply using the intercom at the entrance to a department, a ward or any reserved area, could unlock access for authorized people, as the most natural gesture.

At-home healthcare services appear a perfect fit for voice biometrics and remote authentication,

that can perfectly work through a simple connection to landline or mobile phone networks.

In addition, the combination of voice biometrics and speech recognition unlocks many other opportunities, as voice assistants are entering the healthcare. For instance, patients can use such new devices as conversational interface, to get information and request something needed (see

http://www.healthcareitnews.com/news/special-report-ai-voice-assistants-have-officially-arrived-healthcare). Within the same communication channel, doctors can both authenticate themselves and deploying accurate speech-to-text capabilities to transcribe notes and prescriptions, avoiding illegible handwriting and insufficient documentation of procedures (http://www.rightpatient.com/blog/voice-assistants-technology-healthcare/).

Conclusions

In the healthcare sector, biometrics are generally used in combination with traditional identification approaches (password, PIN, tokens) to authenticate/identify patients and staff, to improve security, cost containment and quality of service. Implementing biometric identification can address both, physical access control for computer/server rooms, hospital ward, and logical access control for login to the workstations and servers providing access to sensitive patient records.

Voice biometrics relies on the most natural form of human communication, requires only cheap hardware for user interface or no new hardware at all thanks to the large diffusion of personal mobile devices. Moreover, voice biometrics allows integration with existing traditional or biometric identification systems, improves mobility and allows at-home healthcare services. All this makes voice biometrics ideal for the healthcare sector in most use cases (except those cases where patients are temporary or permanently unable to speak, that can occur in emergency situations or in some hospital department).

Most of other biometric identifiers, also while successfully employed, require more or less expensive scanners, or cannot easily support remote (such as at-home) authentication), or are invasive with respect to the naturalness of the act of speaking.

Last but not least, voice biometrics technology has reached very high accuracy levels in most operating conditions, and can be successfully integrated in the state-of-art voice-activated personal assistant for many purposes, such as to boost patient engage

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Reflectance Confocal Microscopy in Melanoma and Non-melanoma Skin Cancer

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Abstract

Reflectance confocal microscopy (RCM) is a new technique enabling the visualization of the skin at a quasi-histological resolution, allowing the identification of clues for the diagnosis of skin diseases. The aim of this analysis was to provide new insights into the role of RCM in the diagnosis of skin cancers. Data comes from the most recent literature, taking into account previous essential reported information in this field. The study eligibility criteria were: studies providing update information, focusing on RCM findings in melanoma and non-melanoma skin cancers (NMSC), without restrictions for age, sex, ethnicity. Duplicated studies and single case report were excluded from this study. A search concerning the role of RCM in melanoma and NMSC was performed on the Medline. RCM clues were analyzed for different skin cancers, in particular melanoma and NMSC, in association with clinical, dermoscopic and histopathologic findings. Diagnostic accuracy, sensibility and specificity of the technique were reviewed. Furthermore, some new findings have been described and recent applications have been discussed. The selection of articles was limited in order to provide an up-todate revision. In conclusion, several RCM features were implemented for the diagnosis of melanoma and NMSC, leading to a confocal-based classification in most cases.

Keywords: Microscopy, confocal - Skin neoplasms - Melanoma.

Introduction

Reflectance confocal microscopy (RCM) is one of the latest tools introduced in non-invasive imaging in dermatology. This technique enables in vivo observation at a quasi-histological resolution of the epidermis, dermoepidermal junction (DEJ) and upper dermis, providing horizontal grayscale color images related to refractive index of different tissues and cell structures (Calzavara-Pinton et al. 2008, 1421–30). The highest refractivity is shown by melanin, contained in melanosomes, melanocytes, melanophages and pigmented keratinocytes, followed by structures containing keratin (Rajadhyaksha et al. 1995, 946–52). Noteworthy, cellular contrast in tissue can also be obtained when melanin is present in very small quantities, thanks also to the brightness of some subcellular organelles or surrounding structures (Rajadhyaksha et al. 1999, 293–303). Therefore, RCM can be used for the evaluation of both melanocytic and nonmelanocytic skin lesions.

Several criteria have been described in order to improve diagnostic accuracy (Pellacani, Cesinaro and Seidenari 2005, 979–85). Furthermore, the maximum depth achieved by RCM is approximately 250 µm,3 thus impairing the visualization of the deepest part of skin (Segura et al. 2008, 1311–20; Longo et al. 2013, 58–67). With increasing knowledge, RCM is giving more dynamic insights in the diagnosis of skin cancers.

In order to provide an updated review on the application of RCM, data coming from the most recent literature will be reported and critically revised. A focus on diagnostic accuracy and diagnostic criteria for malignant melanomas and non-melanoma skin cancers (NMSCs) will be provided.

RCM updates for examination of skin cancers

Basic principles of RCM

RCM is a non-invasive method for high-resolution diagnostics of tissue. While conventional microscopes work by using transmitted light technology with thin tissue layers being illuminated from below, RCM works with incident light technology. The skin is illuminated from above with a focused laser thus the reflected light is directed through a pinhole onto a detector so that only signals from a defined horizontal plane are used for high-resolution imaging allows the penetration depth into the skin. This non-invasive technique permits *in vivo* and in real time examinations, allowing microscopic images of skin layers close to the surface and opens up new possibilities for dermatology to monitor both diagnose and progression, in particular in the cases of dynamic changes. It also can be used ex vivo with freshly excised tissue, resembling a frozen section analysis, which is interesting especially for the field of microscopically controlled surgery of skin tumors.

RCM uses a diode laser as a source of monochromatic light that penetrates into the skin and illuminated a small points inside the tissue. The light is reflected, goes through a small pinhole and forms an image in the detector. This small pinhole allows only reflected light from the focal region (confocal) to reach the detector, rejecting all the coming light from out-of-focus plane (Kolm and Braun 2012, 7–10). The reflected light signals from a horizontal plane is then detected, where the refractive index changes. Structures with a higher refractive index appears bright in RCM, such as keratin, melanin and collagen, that result white in RCM.

Notes on the imaging technique

Examinations of a skin lesion is easy. A polymer or glass window is attached to a metal ring ad fixed to the skin with a special adhesive tape to reduce skin movement, where a small amount of immersion oil is applied to the skin. Afterwards, a water-based gel is applied inside the ring. This immersion liquid have refractive index close to that of the epidermis (1.34), thus reducing the spherical aberration of the beam passing through air and therefore allowing sufficient imaging through the epidermis and into the dermis The ring is then magnetically connected to the objective lens housing to stabilize the site of imaging (Kolm and Braun 2012, 7–10; Wurm, Kolm and Ahlgrimm-Siess 2012, 11–19).

RCM images are obtained horizontally from the lesion. Each single image displays a 500μM x 500μM large field-of-view on the screen. It is therefore possible to scan the entire area of the lesion up to 8mm x 8mm. In this way, it is generated a mosaic grid of contiguous horizontal images. This two-diensional composite mosaic is called *VivaBlock*. Additionally, an automated vertical sequence of images, each 500μM field-of-view, can be captured in depth, providing a three-dimensional view of certain area. This is called *VivaStack*. The distance between each section and the section depth can be adjusted. Another useful function is recording a video at 15-20 frames per second, to document dynamic events such as blood flow, or migration of leucocytes. For easier correlation of the macroscopic image and the confocal image, a digital macro camera (Vivacam; Lucid Inc) is linked to the RCM. This camera produces a 5 mega-pixel dermoscopic image of a 10mm field of interest enabling direct viewing of the dermoscopic structures on the RCM monitor. It is possible to navigate within the dermoscopic image and to specify areas for the subsequent RCM viewing and therefore allows choosing the interesting areas in this larger lesion and better asses the borders of a lesion which can be very helpful for surgical planning (Kolm and Braun 2012, 7–10).

RCM acquisition can be carried out with different available tools. The introduction of the handheld RCM device, Vivascope 3000® (Caliber Imaging and Diagnostics, Rochester, NY, USA), permits a better access to difficult anatomic locations and can be used for clinically and dermoscopically equivocal lesions in areas including nasal region, scalp, eyelids, ears and mucosae in order to differentiate benign lesions from skin cancer or for a rapid determination of the nature of the lesion for example melanocytic versus nonmelanocytic (Castro et al. 2015, 1164–69; Fraga-Braghiroli et al. 2014, 933–42; Cinotti et al. 2014, 912–918.e2; García-Hernández et al. 2013, 205256). Clinical applications include the evaluation of recurrences,

assessment of margins for excision like for lentigo maligna (LM)/lentigo maligna melanoma (LMM) and basal cell carcinoma (BCC) and monitoring of non-invasive treatments for AKs (Champin et al. 2014, 247–56; Guitera et al. 2013, 692–98; Longo et al. 2014, 716–724.e1; Ulrich et al. 2010, 15–24).

On the other hand, the wide-probe (Vivascope 1500®, MAVIG GmbH, Munich, Germany) allows a broad non-invasive examination of the epidermis and papillary dermis at cellular-level resolution viewing mosaics (Vivablock®), with a maximum size of 8x8 mm, at epidermis, DEJ and upper dermis level. This probe is the most appropriate for differential diagnosis between nevus and melanoma, because the visualization of the entire lesion is possible and the Vivacam® (Caliber Imaging and Diagnostics, Rochester, NY, USA) (Longo et al. 2015, 31–41) guarantees an overlap with dermoscopic and, recently, also clinical features.

RCM provides substrates for dermoscopic patterns, corresponding to specific histopathological criteria (Pellacani et al. 2008, 1597–1608). This means that, in specific cases, RCM can avoid a skin biopsy while possibly reducing patientrelated costs for excisions. Furthermore, RCM allows the non-invasive exploration of the entire lesion, similarly to what obtained with a shaving biopsy (Wolberink et al. 2013, 985–89).

Diagnostic accuracy and number needed to excise

While histopathology remains the gold standard for the diagnosis of skin cancers, dermoscopy and RCM analysis can improve an early recognition of these lesions.[6] Previous reports described the high sensibility and the increased specificity supporting the analysis of equivocal melanocytic and nonmelanocytic lesions (Guitera et al. 2012, 2386–94; Lovatto et al. 2015, 1918–25), with a better differentiation among skin cancer simulators and interpretation of clinical and pathological correlation (Larre Borges et al. 2014, 833–45; Longo et al. 2012, 799–814; Pellacani et al. 2014, 864–72; Longo et al. 2013, 125–31;). It has been proved that systematic application of RCM diagnostic algorithms showed a specificity ranging between 50% and 70% when evaluating equivocal

lesions (Guitera et al. 2012, 2386–94; Pellacani et al. 2014, 1044–51).

Interestingly, differences in diagnostic accuracy between expert and recent RCM users have been reported (Farnetani et al. 2015, 1075–80), considering that formal training programs have been recently introduced. The mean sensitivity was 88.9% while the mean specificity was 79.3%; experienced RCM users showed higher sensitivity compared with recent users.

Another important issue addressed in the last few years regarded the number needed to excise (NNE). A reduced number of benign lesions excised associated with an improved diagnostic accuracy were obtained with RCM evaluation of lesions eligible for surgical excision because dermoscopically suspected to be malignant or showing dermoscopic changes during the follow-up (Pellacani et al. 2014, 1044–51; Ferrari et al. 2015, 1135–40; Alarcon et al. 2014, 802–8; Stanganelli et al. 2015, 365–71). In particular, in a cohort study enrolling 1005 patients, 423 of which presenting lesions suspected to be malignant, RCM examination reduced the number of lesions for excision to less than one half of benign lesions, passing from a NNE ranging from 8.7 to 29.4 with dermoscopy to 6.8 after RCM evaluation (Pellacani et al. 2014, 1044–51). A previous study (Alarcon et al. 2014, 802–8), performed in a different setting, reported a lower NNE from 3.73 to 2.87.

Dysplastic nevi

Dysplastic nevi represent major risk factors for melanoma and they are challenging lesions both clinically and histopathologically (Elder 2010, 112–20; Duffy and Grossman 2012, e1-16). A simplified algorithm was developed by Pellacani et al. (Pellacani et al. 2012, e109-121) in order to differentiate nevi, dysplastic and nondysplastic, from melanomas. 60 lesions among which 19 nondysplastic nevi, 27 dysplastic nevi and 14 melanomas were analyzed by RCM and histopathology.

RCM criteria found in melanocytic lesions are presented in Table I.

Upon RCM, dysplastic nevi were usually characterized by a ringed and meshwork patterns with irregular junctional nests showing short interconnections or atypical junctional cells mostly located in the center of the lesion. In de-

tails, the presence of cytologic atypia in association with atypical junctional nests (showing short interconnections or characterized by nonhomogeneous cellularity) was suggestive of dysplasia, while widespreading pagetoid cells scattered in the epidermis or atypical cells diffused throughout the DEJ associated with nonedged papillae were tipically found in melanoma (Pellacani et al. 2012, e109-21)

A focus on pink and nodular lesions

Many studies regarding pigmented lesions, showing bright components due to the high content of melanin, have been performed, while hypopigmented/amelanotic lesions were not so extensively investigated. A stepwise approach was described in order to study hypopigmented/amelanotic lesions (Longo et al. 2015, 31–41). Initially, the distinction between inflammatory versus skin cancer has to be formulated. Then, RCM evaluation can be helpful in determining the nature of the lesion, underlining the importance of identifying specific morphological clues in order to classify nonpigmented skin neoplasms into melanocytic versus nonmelanocytic and benign versus malignant. More specifically, the presence of a nested melanocytic proliferation at the DEJ or dermis level allowed to ascribe a given lesion as melanocytic; the presence of basaloid bright tumour islands was a key RCM feature for the diagnosis of BCC; and an epidermal disarrangement associated with small demarcated papillae was suggestive for the diagnosis of SCC (Longo et al. 2015, 31–41).

The second issue, depth exploration, was analyzed in a retrospective study involving 140 nodular lesions. Nodular neoplasias belonging to both melanocytic (among which 23 "pure" nodular melanomas [NMs], nine melanoma metastasis) and nonmelanocytic tumours (among which 28 BCCs and six invasive SCCs) have been explored, concluding that RCM was a valuable tool for nodules because, in many cases, a thinned epidermis allowed a good visualization of peculiar diagnostic structures located in the superficial dermis (Longo et al. 2013, 58–67). The key concept of this study was that cases characterized by the absence of criteria should lead to prompt excision because a deep malignant tumour proliferation cannot be assessed

and thus melanoma diagnosis cannot be ruled out.

RCM updates for examination of melanoma

Melanoma is one of the tumors with the highest rising in incidence. Only an early detection of melanoma can led to a better prognosis (Garbe and Leiter 2009, 3–9).

The original histopathologic classification of melanoma is based on growth pattern and biologic behavior and it included LM/LMM, superficial spreading (SSM) and NM. Then, other types of melanoma have been added to this classification (Clark et al. 1969, 705–27).

Superficial Spreading Melanoma (SSM)

SSM is the most common type of melanoma in Caucasian (Jemal et al. 2008, 71–96). It is characterized by a radial growth in the initial phase, followed by vertical proliferation after invasion. A 3-steps model of progression in SSM has been described (Scope et al. 2008, 1644–49). The first step was the proliferation of single cells or melanocyte nests along the DEJ occurring in the rete ridges, in the suprapapillary plate and in suprabasal layer of epidermis, then a remodeling with a progressive flattening of DEJ associated with inflammation, angiogenesis and fibroplasia followed by dermal invasion. This means that to each step corresponded an increased degree of atypia. Pagetoid cells, mild to moderate cytological atypia and non-edged papillae can be described in superficial lesions (Gareau et al. 2010, 61713). With increasing thickness, epidermal disarrangement, cell pleomorphism, progressive papillary infiltration with bright cells or cerebriform melanocyte nests can be added to the list of features observed (Pellacani, Cesinaro and Seidenari 2005, 469–74).

In particular, considering epidemiologic and RCM characteristics, SSM included: pagetoid and solar melanoma (Longo, Casari and Pellacani 2012, 151–78). Pagetoid melanoma was described in adults with a history of intermittent solar exposure and several nevi. Upon dermoscopy, reticular, globular, multicomponent or nonspecific pattern could be identified. Histologically, this tumor is typified by an intraepidermal growth, corresponding to RCM findings of pagetoid infiltration (Pellacani, Cesinaro and Seidenari 2005, 532–37) of the epi-

dermis that showed an atypical honeycomb or cobblestone or fully disarrayed pattern. Pagetoid cells were mainly roundish and distributed throughout the lesion or located at the periphery (Figure 1A, B). When centrally located, a differential diagnosis with Spitz, congenital, traumatized and dysplastic nevi should always be considered (Pellacani et al. 2009, 236–47). DEJ was predominantly represented by meshwork with edged and nonedged papillae with admixed atypical cells. Going deeper to the upper dermis, melanocytic homogeneous nests or, in over the half of cases, dense and sparse nest were prevalent. Inflammation presented as plump bright cells and bright particles (Longo, Casari and Pellacani 2012, 151–78).

Melanoma on sun-damaged skin is located by definition in the most sun-exposed areas of patients with a low nevus count. Dermoscopically regression features such as white combined with blue-gray color and/or peppering. Histologically, atypical melanocytes in the basal layer and a lymphocytic infiltrate were commonly presented in the superficial portions of the dermis while the surrounding skin showed photodamage. Peculiar RCM findings were pagetoid cells with variable shape, usually dendritic, mainly located around the hair follicle, in the context of an atypical or disarranged epidermis. At the DEJ, ringed or meshwork or uneven pattern could be observed in association with atypical cells. Upper dermis was predominantly characterized by solar elastosis and inflammation. Polycyclic papillary contours and bulbous projections, sometimes detectable at the periphery of the lesion could led to a misdiagnosis with a benign lesion, underlying the importance of a fully evaluation of the lesion (Longo, Casari and Pellacani 2012, 151–78).

Nodular Melanoma (NM)

NM is the most aggressive type of melanoma (Chang, Karnell and Menck 1998, 1664–78). A difference should be made between vertical growth phase in a context of radial growth phase of SSM and "pure" NMs, these latter representing a separated subgroup of melanomas (Segura et al. 2008, 1311–20).

Pigmented NMs usually appeared as symmetric lesions, with a homogeneous blue pigmentation, showing also a blue-white veil, pink and/or black color and milky-red/pink areas with large-diameter vessels predominantly located in the periphery, with pure NMs showing less dermoscopic features than SSMs with a vertical growth phase (Menzies et al. 2013, 699–709).

Table 1. RCM criteria for the evaluation of melanocytic lesions

RCM criteria	
Epidermis	
Regular honeycombed pattern	Large polygonal cells with dark nuclei and bright cytoplasm and cell borders
Regular cobblestone pattern	Small polygonal cells with refractive cytoplasm separated by a less refractive border
Atypical honeycombed pattern	Irregularity in size of the cells and thickness of the contour within a honeycombed structure
Atypical cobblestone pattern	Irregularity in size and/or refractivity of the cells within a cobblestone structure
Broadened epidermal pattern	Polygonal cells with demarcated, bright borders and black nuclei
Epidermal disruption	Disarray of the normal architecture of superficial layers with unevenly distributed bright granular particles and cells, in absense of honeycombed or cobblestone pattern
Acanthosis	Prominent bright epidermis intermingled with papillae
Ulceration	Dark areas, usually with sharp borders and irregular contours, filled with amorphous material and/or clotted bright small particles
Pagetoid cells	Large roundish nucleated cells, twice the size of basal keratinocytes, with a dark nucleus and bright cytoplasm, with different number and distribution
Dendritic pagetoid cells	Large cells with bright cytoplasm and well-demarcated dendrites, as well as clearly visible dendrites connected to the cell
Roundish pagetoid cells	Large bright cells with well outlined border and dark nucleus within the epidermis, represent the most common finding for melanoma diagnosis
Hyporeflective pagetoid cells (HPCs)	Large cells, round to oval in shape, appearing as well-demarcated structures within the epidermal layer
Striking pleomorphism	Variability of the aspect of pagetoid cell and/or the presence of cells with bizarre shapes
Dermoepidermal junction (DEJ)	
Ringed pattern	Densely packed bright rings corresponding to papillae surrounded by a rim of small bright cells sharply contrasting with the dark background
Junctional nests	Compact, round to oval bright cell aggregates, connected with the basal layer of the epidermis
Sheet-like strucure	Cells distributed at the transition of the DEJ showing loss of dermal papillae not aggregated in clusters but closely distributed in the same plane with the loss of dermal papillae
Edged papillae	Dermal papillae demarcated by a rim of bright cells, appearing as bright rings sharply contrasting with the dark background
Non-edged papillae	Dermal papillae without a demarcating bright rim at the DEJ
Atypical cells	Large cells showing a bright cytoplasm with clearly outlined borders and sharply contrasted dark nucleus inside, roundish to oval in shapes, sometimes presenting dendritic-like structures, showing different number and distribution
'Medusahead-like' structure.	Dendritic and pleomorphic cells aggregated into clusters budding from the hair follicle
Upper dermis	
Dermal dense nests	Compact aggregates with sharp margin in which outline of individual cells is indiscernible or similar in shape, size and refractivity
Dermal dense nonhomogenous nests	Compact cell aggregates showing non homogeneity in cell morphology and reflectivity
Dense and sparse dermal nests	Cell aggregates with irregular, discohesive margins showing isolated nucleated cells at the periphery
Cerebriform clusters	Confluent amorphous brain-like aggregates of low reflecting cells exhibiting granular cytoplasm without evident nuclei and ill-defined borders, showing a fine hyporeflective "fissure" like appearance
Striking pleomorphism within nests	Nonreflecting structures with a well-demarcated border, containing isolated round to oval cells with dark nucleus and reflecting cytoplasm, with bizarre shapes
Inflammation	Large irregularly shaped bright cells with ill-defined borders and usually no visible nucleus which can be visible within dermal papillae
Collagen	Bright elongated fibrillar structures with no cellular component, no visible nucleus and movement, distributed within the dermis, arranged in a reticulated network or as bright bundles

Histologically, a flattening of the epidermis and eventual ulceration overcoming the nodular component could be found whereas a cohesive nodule or smaller nests of malignant melanocytes with a pushing or expansive pattern of growth at the dermis level were found. Upon RCM, "epidermal consumption" was detected while acquiring RCM image stacks in NMs. This feature was characterized by a rapid of transition from epidermal to dermal compartment, due to the thinning of the epidermis down to two to three layers of keratinocytes (Hantschke, Bastian and LeBoit 2004, 1621–25).

Epidermal layers were characterized by regular honeycombed or, sometimes, polygonal cells with demarcated, bright borders and black nuclei forming a broadened honeycombed pattern. Pagetoid cells, with either a roundish or dendritic shape, were identified in vertical growth phases whereas in NMs the pagetoid

infiltration was not detectable or focally represented.

Ulceration represented a common finding, appearing as dark areas, usually with sharp borders and irregular contours, filled with amorphous material and/or clotted bright small particles. Ulcerations covering over than one half of the surface of the lesion hampered penetration of RCM light and visualization of underlying structures. At the DEJ, typical dermal papillae were absent and the basal layer was composed of pleomorphic cells, arranged in sheet-like structures.

Within the dermis, cerebriform melanocyte nests with prominent cellularity and moderate inflammatory infiltrate with the absence of regression were identified. Cerebriform nests, showing up as hyporefrective aggregates of small cells outlined by bright collagen septae, were considered highly specific of malignancy and suggestive of invasion of the deepest part of dermis in NMs (Figure 1C, D) (Segura et al. 2008, 1311–20). Interestingly, RCM features found within dermal component of nodules arising from a SSM or to pure NMs were not distinguishable.

Furthermore, vascularization was found to be an important clue at this stage of invasion. Convolute and tortuous vessels, in proximity of tumoral proliferation or even crossing malignant cell growth, were frequently decribed. Live imaging unrevealed the presence of vessels with large caliber and fast blood flow.

Recently, Longo et al. (Longo et al. 2015, 31–41) described the role of RCM in the evaluation of 140 nodular lesions including NMs, melanoma metastasis and other benign and malignant skin lesions.

Considering that the nodular portion of NMs shared morphologic findings with metastasis, these two entities where grouped in the discriminant analysis, highlighting that widespread pagetoid distribution, many atypical cells and cerebriform nests were peculiar elements for the diagnosis of melanocytic malignancy.

Whitmore, confocal microscopy reached 96.5% sensitivity and 94.1% specificity in the diagnosis of NMs and melanoma metastasis versus all other nodular lesions, regardless of the limited laser depth penetration of RCM. Limitations were represented above all by wide ulcerations or marked hyperkeratosis (Longo et al. 2015,

31–41). This means that, once again, biopsy and histopathological examination should always be performed in doubtful cases.
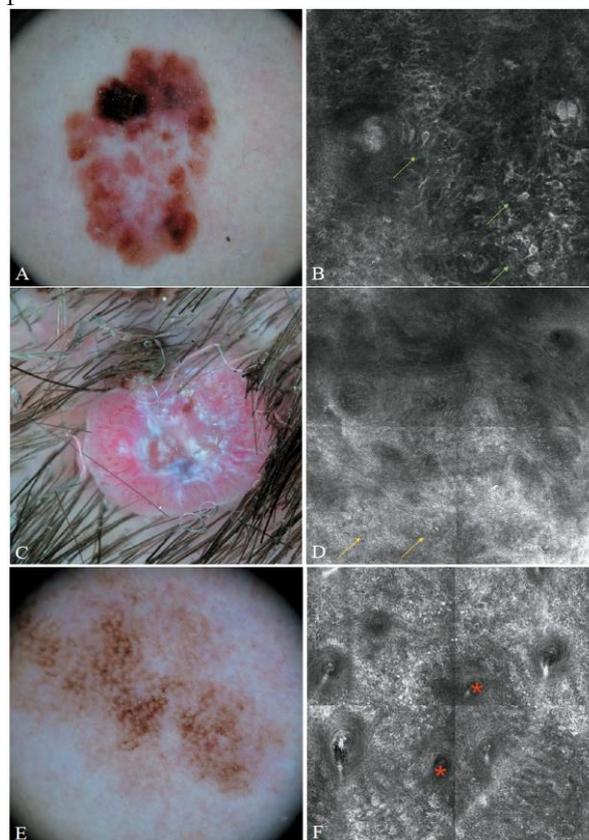
Figure 1. Dermoscopic and confocal pictures of melanomas. A) Dermoscopic image of superficial spreading melanoma. It presents multi component pattern with central area of regression, eccentric hyperpigmentation, inverse network; B) confocal image of melanoma. Several roundish pagetoid cells (green arrows) within the epidermis with a consumption of he epidermis itself; C) dermoscopic image of nodular amelanotic melanoma showing irregular vessel, central ulceration; D) confocal image of nodular melanoma showing the presence of cerebriform nest (yellow arrows) in the dermis. These nests are rarely notices in melanoma because they are too deep in the dermis but they are typical of more invasive melanoma; E) dermoscopic image of lentigo maligna (LM) of left cheek. A hyperpigmentation around follicular openings can be noticed; F) confocal image of LM with numerous dendritic pagetoid cells around follicular openings (red asterisks). This is a typical sign of LM (Guida et al. 2015, 547–63).

Lentigo Maligna (LM) / Lentigo Maligna Melanoma (LMM)

LM and LMM are melanomas mainly occurring on the face and other sun-exposed areas. Dermoscopy identified several peculiar aspects like asymmetrical follicle pigmentation, annular granular pattern, pigmented rhomboidal structures and/or obliterated hair follicles (Pralong et al. 2012, 280–87). RCM examination of LM/LMM revealed a significant similarity to solar SSM. Epidermal disarrangement with the presence of numerous round or dendritic ple-

omorphic cells, distributed in all of its layers, was reported (Langley et al. 2006, 88–97). An important clue was the presence of atypical cells grouping around the hair follicle (Langley et al. 2006, 88–97; Tannous et al. 2002, 260–63). In the papillary dermis, the presence of melanophages and reticulated bright collagen bundles, ascribable to solar damage were observed. Nests of atypical and nucleated melanocytes could also be seen and these structures corresponded, coming back to dermoscopy, to globules (de Carvalho et al. 2016, 878–80).

In a recent study (de Carvalho et al. 2015, 128–33), RCM examination of 60 lesion revealed that the most relevant differences between the nonmelanoma skin neoplasms and melanomas involved the presence of atypical cells both at th junction and spreading upwards in a pagetoid fashion, usually with a dendritic morphology (Figure 1E, F). Furthermore, a meshwork pattern was observed in LM/LMM, compared with the most common ringed pattern in association with polycyclic contours in benign lesions. An important clue, known as 'Medusahead-like' structures, characterized by elongated buddings bulging from the hair follicle and populated by dendritic/pleomorphic cells, was found in 11 of 30 LMs of the case series analyzed, usually in relation to asymmetric follicular pigmentation and/or around hyperpigmented follicles, whereas folliculotropism of atypical cells was detected in one half of cases. Another important aspect concerned the helpfulness of RCM in the analysis of hyperpigmented areas, suspicious for malignancy (Tran, Wright and Cockerell 2008, 852–71). In fact, when evaluating each dermoscopic pattern in lesion with different biological nature, RCM substrates helped in providing the correct diagnosis avoiding a skin biopsy (de Carvalho et al. 2015, 128–33).

Noteworthy, suggestive RCM features for LM can be found in early melanomas, providing differential diagnosis from solar lentigo, seborrhoeic keratoses and lichen planus-like keratosis (de Carvalho et al. 2015, 128–33). Finally, different attempts have been made in order to define margins of LM/LMM more accurately using RCM (Champin et al. 2014, 247–56; Guitera et al. 2013, 692–98). Preoperative mapping is extremely important because these lesions often show ill-defined margins, not easily detectable with the naked eye and with the help of dermoscopy. The assumption is that a careful preoperative mapping guiding surgical excision could led to a reduction of local recurrences. Intuitively, the analysis of LMs/LMMs with the handheld RCM device Vivascope 3000 can be useful in difficult-toexplore sites and in lesions bigger than 8x8 mm.

Amelanotic melanoma

The diagnosis of amelanotic melanoma (AM) has been challenging clinically and instrumentally (Guitera et al. 2013, 692–98). RCM features described for SSM and NM can also be applied for AMs (Longo et al. 2015, 31–41). Nevertheless, considering that melanin and melanosomes usually create a source of contrast, (Stoecker and Stolz 2008, 1207–10) the identification of clues became fundamental. Interestingly, Losi et al. (Losi et al. 2014, 48–54) detected a new confocal feature called hyporeflective pagetoid cells (HPCs). HPCs were described as large cells, round to oval in shape, appearing as well-demarcated structures within the epidermal layer. At a higher magnification, these structures, with a particular shape and size, presented a dark round central area corresponding to the nucleus, within a dark grey grainy material corresponding to the cytoplasm. A difference should be operated between flat and papular/nodular lesions. Flat AMs were characterized by honeycombed pattern in association with HPCs, single or organized in nests, and dendritic-shaped particles corresponding to melanocyte or Langerhans cells (Hashemi et al. 2012, 452–62). At the DEJ level, different architectural patterns were identified: nonedged dermal papillae, nonspecific pattern with a loss of dermal papillae, due to the flattening of the DEJ or a meshwork pattern with focal or wide spreading irregular junctional nests composed by atypical melanocytes (Pellacani et al. 2014, 414–18). In upper dermis melanocyte nests were usually less bight than nests belonging to pigmented tumors.

In palpable-to-nodular AMs, honeycombed epidermal pattern with a more evident brightness of the intercellular borders of keratinocytes was observed.

HPCs were found more commonly in SSM with a nodular component than in pure NMs, like pagetoid cells in pigmented NMs (Longo et al. 2013, 58–67).

As previously described for pigmented NMs, ulceration was a common finding also in nodular AMs while cerebriform nests, although rare and presenting a weak reflectivity, represented the most important clue for the diagnosis of NM (Segura et al. 2008, 1311–20).

In order to validate this feature, a retrospective study has been performed, including 20 amelanotic MMs and controls represented by 10 melanocytic nevi, 20 hypo/nonpigmented nonmelanocytic

lesions and 20 pigmented melanomas. HPCs were abundantly found in 85% of AMs. They were also described in Spitz nevi. In these cases they were correlated with pagetoid infiltration of hypomelanotic melanocytes on histopathology. This is an important finding when considering that few HPCs were also highlighted in nonmelanocytic lesions (three SCCs, two seborrhoeic keratoses and one BCC), corresponding to enlarged or dyskeratotic keratinocytes by histopathology (Losi et al. 2014, 48–54).

Mucosal melanoma

This is a rare form of melanoma. Although most mucosal pigmented macules are benign, it can be clinically and dermoscopically challenging to rule out an early melanoma, above all when considering that most cases of malignancy in oral mucosa and anogenital are relatively advanced at diagnosis.

Early detection and appropriate treatment significantly increases survival. RCM findings for this type of melanoma included: roundish cells, a high density of atypical dendritic cells and intraepithelial bright cells in a case series including labial or genital pigmented macules among which 10 macular melanomas (Debarbieux et al. 2014, 1276–84).

Toward a confocal-based classification of melanoma

A previous report 4 described a RCM-based diagnostic algorithm consisting of 2 major and 4 minor criteria to evaluate the degree of atypia of melanocytic lesions. Major criteria (scoring two points each) included non-edged papillae and the presence of atypical melanocytes. Minor criteria (scoring one point each) included pagetoid cells in the epidermis, wide spreading pagetoid cells, cerebriform melanocytic clusters, and individual, nucleated melanocytes within dermal papillae. A score of ≥ three points led to a confocal-based diagnosis of melanoma.

A web-based multicenter study involving RCM users from 6 different countries, evaluated characteristic features of 55 melanocytic nevi, 20 melanomas and other nonmelanocytic skin neoplasms. The discriminant analysis of these lesions identified three important features for melanoma diagnosis. Pagetoid cells spreading within epidermis, atypical cells at the DEJ and irregular architecture of epidermis; these latter showed a low interobserver reproducibility (Farnetani et al. 2015, 1075–80).

Recently, Pellacani et al. (Hashemi et al. 2012, 452–62) have described a confocal- based classification. The study included 100 melanomas, of which 11 LMs, nine LMMs, 74 SSMs (15 of which in situ) and six NMs. Dendritic and round cell, dermal nest, combined and non-classifiable melanomas have been described, depending on prevalent RCM features.

Dendritic cells were found in large melanomas showing a pigmented network in subjects with a low number of atypical nevi. Dendritic cells characterized a slow-growing group of melanomas, described by Argenziano et al. (Argenziano et al. 2010, 267–73) in which round cells and dermal nests could also be found with the progression of the disease. This observation allowed to formulate the hypothesis that the initial growth of melanoma could be represented by intraepidermal proliferation of dendritic melanocytes that, when de-differentiating, could leave the place to cells with a roundish shape and invasive capability (Zalaudek et al. 2008, 1375–79). LMs and SSMs were predominantly observed in this group of melanomas (Pellacani et al. 2014, 414–18).

Round cells were commonly associated with thicker but smaller melanomas compared to dendritic ones, occurring in patients with many atypical nevi. The degree of invasiveness was variable. The next step for melanoma progression could be exemplified by a further cell de-differentiation in order to create less cohesive cells aggregated into dense-andsparse clusters and/or cerebriform nests (Pellacani, Cesinaro and Seidenari 2005, 469–74).

Dermal nests typified NM, characterized by a rapid pattern of growth, a deep dermal invasion, limited epidermotropism and poorly differentiated melanocytes, (Zalaudek et al. 2008, 1375–79) while combined melanomas were the biggest ones. Nonclassifiable type melanomas showed ringed architecture with few atypical cells, usually roundish; these were supposed to represent an early phase of round cell melanomas (Pellacani et al. 2014, 414–18).

Finally, Longo et al. (Longo et al. 2013, 941–45), used RCM in support to dermoscopy and histopathology, and described a new melanoma entity called "nested melanoma of the elderly", characterized by the presence of a clod pattern in which compact nests with variable atypia.

RCM for the examination of NMSC

Skin cancer is the most commonly diagnosed cancer in the Caucasian population, with rapid further increasing incidence rates (Rubin, Chen and Ratner 2005, 2262–69). SCC and BCC are considered as NMSCs. The incidence ratio between those two NMSC types is approximately 1:4, while in immunosuppressed patients this ratio is inverted (Ridky 2007, 484–501; Lomas, Leonardi-Bee and Bath-Hextall 2012, 1069–80). RCM criteria for NMSCs are summarized in Table II.

Table 2. RCM criteria for the diagnosis of non-melanocytic skin cancer (NMSCs)

RCM criteria	
Basal cell carcinoma	
Tumor islands	Round to oval, cord-like or lobulated structures at the level of DEJ or superficial dermis that can be either darker than the surrounding epidermis or dermis ("dark well-demarcated structures silhouettes") or bright well-demarcated structures
Polarization of nuclei (streaming)	Cells within the tumor islands, or overlying basal or spinous keratinocytes, display nuclei that are elongated and distorted into alignment along the same axis
Dark cleft (clefting)	Dark slit-like space observed between tumor island and surrounding dermis
Dendritic cells	Bright delicate, dendritic structures within bright tumor islands or in the overlying epidermis
Plump-bright cells	Oval to stellate cells with indistinct borders and without apparent nucleus in the dermis
Canalicular blood vessels	Thickened, elongated or tortuous dark structures, oriented parallel to the skin surface, containing moving small, round bright structures (white blood cells)
Fenestrated pattern	Tumor islands or cords with palisading cells at the periphery, which outline hyporefractile 'holes' corresponding to the fibrous stroma
Actinic keratosis and squamous cell carcinoma	
Parakeratosis	Individual highly-refractile round cells in the stratum corneum
Scale (hyperkeratosis)	Increased thickness of stratum corneum seen as refractile amorphous material
Irregular (atypical) honeycomb pattern	Abnormal pattern of the spinous–granular layers formed by bright cellular outlines
Solar elastosis	Moderately refractive lace-like material adjacent to collagen bundles
Inflammatory infiltrate	Small highly refractile cells within the epidermal layers and the superficial dermis
Round blood vessels	Dilated blood vessels within the dermal papillae that run perpendicular to the horizontal RCM plane of imaging.

Confocal patterns and characteristics of BCC

BCC is the most common skin cancer worldwide, mainly affecting fair-skinned adults. BCC is a slow-growing tumor that rarely metastasizes. It is one of the most studied skin cancers upon RCM, and this can be related to its high incidence. Clinically, BCC is characterized by pink to red-brown patches or papules, central erosion or ulceration with or without the presence of crusts, a pearly shine and a raised border with the presence of telangiectasias (Webber et al. 2011, 179–85).

Some differences in diagnostic accuracy have been evaluated in a recent study involving 54 lesions of which 45 histologically proven BCCs. Castro et al. (Castro et al. 2015, 1164–69) highlighted a high positive predictive value obtained with both RCM probes, while a higher negative predictive value was associated with the use of the traditional wide probe, probably because its broader field-of-view allows a more exhaustive search for BCC criteria.

Several dermoscopic patterns were identified but they will be taken into account for BCC subtype classification that follows. On histopathology, most BCC subtypes show aggregates of atypical basaloid cells with peripheral palisading of nuclei, stroma with fibroplasia, and frequently clefting between tumor aggregates and stroma.

González et al. firstly described five relevant criteria for the confocal diagnosis of BCC, which were later validated in a larger study (González and Tannous 2002, 869–74; Nori et al. 2004, 923–30). These criteria included: elongated monomorphic nuclei, polarization of these nuclei along the same axis, a prominent inflammatory infiltrate, increased dermal vasculature and pleomorphism of the overlying epidermis.

Later on, further descriptors were added, such as the presence of tumor islands and cords considered the RCM hallmark of the tumor. These structures were characterized by a bright outline and clearly defined shape, especially in the presence of pigmentation.

However, they were usually clearly visible also in hypopigmented tumors, although less contrasted.

Dark silhouettes represented a different morphologic presentation of BCC tumor islands, corresponding to hypopigmented tumor proliferation visible as a dark, footprint-like shadow in a context of bright compact collagen (Figure 2A, B) (Agero et al. 2006, 638–43; Segura et al. 2007, 883–86; Scope, Mecca and Marghoob 2009, 106–7; Braga et al. 2009, 230–41; Segura et al. 2009, 216–29; Ulrich et al. 2011, 190–95; Casari et al. 2011, 406859).

Additionally, the presence of dendritic cells in BCC nests was correlated with melanocytes, typically found in pigmented BCCs where they appeared as long dendritic shaped cells entrapped inside the tumor islands (Segura et al. 2007, 883–86). Along with melanocytes, the pigmentation upon RCM was related to the presence of inflammatory infiltrate showing up as bright spots or to ill-defined plump bright cells corresponding to melanin-rich melanophages (Figure 2C, D). (Guitera et al. 2012, 2386–94).

Blood flow was typically altered in BCC and was described as an elongation of blood capillaries, which were increased in number and size. In a recently published large prospective study, Guitera et al. (Guitera et al. 2012, 2386–94) introduced a new concept called "epidermal shadowing", described as a large dark featureless area disrupting the epidermis due to en face clefting of the underlying tumor nests.

RCM subtypes of BCC

The identification of different subtypes of BCC is important in order to choice the most appropriate treatment and to determine surgical excision margin.

BCC can be divided into 3 main subtypes based on the histopathologic growth pattern: nodular (nBCC), infiltrating (iBCC) and superficial (sBCC). Longo et al. (Longo et al. 2014, 716–724.e1) presented a study to identify specific criteria to differentiate subtypes of BCC thanks to the retrospective analysis of 88 lesions, including 44 sBCCs, 22 nBCCs, and 22 iBCCs.

Firstly, different BCC subtypes were differentiated dermoscopically being sBCC characterized by fine telangiectasias, multiple small erosions, and structures corresponding to dermoepidermal pigmentation. In contrast, the detection of ovoid nests, arborizing vessels and large ulcerations were associated with nBCCs or iBCCs.

iBCC has been reported to have a peculiar dermatoscopic pattern, consisting of shiny white-red structureless areas and arborizing vessels of a smaller caliber and less tendency to branch into finer capillaries compared to those seen in nBCC tumors (Lallas et al. 2014, 303–11). Upon RCM, the presence of cords connected to the epidermis was significantly associated with sBCC. nBCC was primarily typified by the presence of large tumors nests.

Furthermore the presence of clefting was much more frequent in nBCC compared to sBCC and iBCCs.

Finally, although increased vascularization was detected in all subtypes, the caliber of vessel was larger in nBCC compared to sBCC and iBCC. iBCC was characterized, upon RCM, by the presence of dark silhouettes and abundant bright compact collagen.

Moreover, iBCC was the most common diagnosis in presence of dark silhouettes and in absence of small tumor islands, big tumor islands, and cords connected to the epidermis. Peripheral palisading was present in the majority of tumors of all subtypes, while inflammation was less frequently observed (Horn et al. 2008, 620–25).

A previous study (Peppelman et al. 2013, 255–62) involved also micronodular (mnBCC) and mixed-type variants. 27 patients with 43 biopsied lesions with a histopathological diagnosis of BCC were included. Of these, 23 were sBCC, 11 nBCC, three mnBCC and six mixedtype BCC.

Tumor nests with peripheral palisading, branch-like structures, fibrotic septa and increase in vascular caliber were the main RCM features for nBCC and mnBCC. Then size, shape and location of the tumor nests allowed a differentiation between nBCC and mnBCC. Solar elastosis and the location of the tumor nests just below or in connection with the basal cell layer characterize sBCC. Criteria for iBCC were not easily detectable in this study because mixedtype but not "pure" iBCC were included (Peppelman et al. 2013, 255–62).

Another variant that should be mentioned is fibroepithelioma of Pinkus (FeP), representing an unusual form of basal cell carcinoma, which may clinically mimic a range of benign skin tumors that are not routinely excised. 20 published cases of FeP reported in the literature

were reviewed, suggesting that dermoscopy and RCM could help the clinical diagnosis and, consequently, management of FeP (Reggiani et al. 2013, 207–11). On characterhistopathology, FeP showed tumor islands and anastomosing strands of basaloid, often palisading cells, included in a fibromatous stroma. Upon RCM, the analysis of 9 cases (6 nonpigmented and 3 pigmented) revealed the presence of a "fenestrated pattern", consisting of tumor islands or cords with palisading cells at the periphery, which outline hyporefractile 'holes' corresponding to the fibrous stroma (Longo et al. 2012, 556).
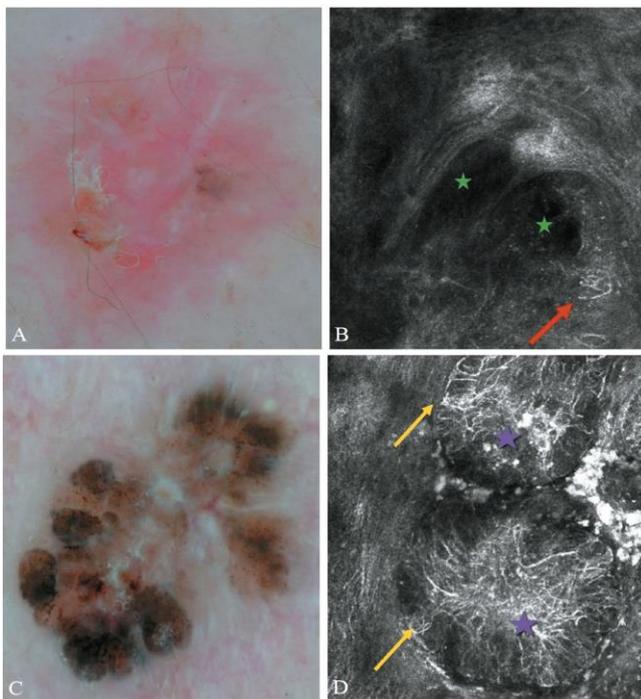
Figure 2. Dermoscopic and confocal pictures of basal cell carcinomas (BBCs). A) dermoscopic image of invasive BCC, lightly pigmented. There are multiple ulcerations, fine multiple telangiectasia and blue-grey dots; B) confocal image (square 0.5x0.25) showing typical features enabling the diagnosis: dark silhouette (green star) and bright filaments corresponding to melanophages (red arrow) that create the light pigmentation visible dermoscopically; C) dermoscopic image of nodular pigmented BCC: grey-blue ovoidal nest, leaf-like maple; D) confocal image of multiple tightly packed (violet stars) and numerous melanophages (yellow arrows). The major number of melanophages is related to the pigmentation more evident in this BBC (Guida et al. 2015, 547–63).

Confocal patterns and characteristics of AK

AKs represent the earliest stage in the development of SCC and relevant biomarkers for individuals at risk for development of invasive SCC.

The main risk factors are fair skin, intermittent or prolonged exposure to UV radiation, genetic predisposition, and immunosuppression (Ulrich et al. 2008, 610–19). Usually the diagnosis of AK is based on clinical examination, but biopsies are routinely performed in order to rule out invasive SCC or other skin diseases like porokeratoses, seborrheic keratoses, basal cell carcinoma or Bowen's disease. Dermoscopy has recently been applied for the diagnosis of AK, the classic features include the presence pink-to-red "pseudonetwork" surrounding the hair follicles, scale, fine and linear

vessels surrounding the hair follicles and keratotic plugs within the hair follicles (Horn et al. 2008, 620–25). Histopathological features included: proliferation of atypical keratinocytes AKs, starting at the basal cell layer, inducing an architectural disarray due to basal crowding of keratinocytes with different degrees of involvement of epidermis. Mitoses and nuclear pleomorphism represented a common finding together with parakeratosis alternated with orthokeratosis at the follicle ostia in the stratum corneum, solar elastosis and inflammation in the dermis.

Confocal laser microscopy could be useful in order to perform the correct diagnosis. First of all, keratinocytes in the stratum corneum appeared detached from one another and are seen as highly refractive polygonal structures. Moreover, nuclear retention, corresponding to parakeratosis in histology, was visualized as dark round structures in the center of the corneocytes. RCM images showed an atypical honeycomb pattern (disruption of the characteristic honeycomb pattern) and cells with dark nuclei of irregular shapes and sizes (Figure 3A, B). Images of the superficial dermis showed different degrees of solar elastosis, which is visualized as bundles of lace-like collagen (Horn et al. 2008, 620–25).

Confocal grading of AKs

Several options are nowadays available for the treatment of AKs. This means that specific patterns corresponding to different grades and subtypes may aid to choose the most appropriate treatment and in monitoring of the response to the therapy. Interestingly, preliminary confocal microscopy data based on keratinocyte atyp-

ia allowed the creation of a grading of AK. Grade 1 AK presented focal areas of atypical honeycombed pattern at the level of the stratum spinosum, intermingled with areas of preserved, typical honeycombed pattern. In grade 2 AK, the atypia of keratinocytes was more diffuse, involving the stratum spinosum and granulosum. Keratinocytes presented a marked atypia, with different sizes and shapes of the cells. Grade 3 AK was characterized by a markedly atypical honeycombed pattern with areas of partial disruption of the normal epidermal layers, defined as a disarranged pattern. Pleomorphic keratinocytes showed a wide variability in cellular size and shapes, and irregular intercellular keratinocyte connections were detected (Zalaudek et al. 2014, 80–87).

Confocal patterns and characteristics of SCC

SCC is a common skin tumor derived from epidermal keratinocytes. It typically presents as a red scaling plaque, with or without ulceration. SCC is complicated by its metastatic potential (Soter, Wilkinson and Fitzpatrick 1973, 296–302). Dermoscopy features of SCC were dotted or glomerular vessels, when present, and a scaly surface that, when too wide, could impair the visualization of vessels (Zalaudek et al. 2004, 1112–16).

Histopathologically, crowded, enlarged, and pleomorphic nuclei, dykeratosis, and parakeratosis were typically found in SCCs (Ackerman and Mones 2006, 9–22). Bowen disease (BD) is SCC in situ that is histologically characterized by a proliferation of atypical pleomorphic keratinocytes throughout the epidermis.

RCM features of keratinocytic atypia have been described in recent studies (Horn et al. 2008, 620–25; Ulrich et al. 2008, 610–19; Rishpon et al. 2009, 766–72), but nowadays no detailed description of RCM features of BD exists in the literature.

Ulrich et al. (Ulrich et al. 2012, 451–53) recently published a study of 10 BD. The most common confocal findings were disruption of the stratum corneum, an atypical honeycomb pattern in the epidermis with a greater degree of architectural disorder and cellular atypia than in AK (Figure 3C, D), S-shaped blood vessels in the center of the dermal papillae, and two types of characteristic targetoid cells. The first type were large cells with a dark center, a bright rim, and a dark halo, and the second type were large cells with a bright centerand a dark halo. Cells are thought to correspond to the different degrees of dyskeratosis seen by conventional histology. Other features observed were parakeratosis, multinucleated cells, and solar elastosis.

In 2009 Rishpon et al. (Rishpon et al. 2009, 766–72) published a study of the confocal characteristics of 38 clinically suspected SCC lesions. The features identified were an atypical honeycomb or disarranged pattern in the epidermis (Figure 3E, F), rare round cells with nuclear atypia in the stratum spinosum and stratum granulosum, and round blood vessels crossing the dermal papillae.

RCM images of the stratum corneum typically revealed bright amorphous structures corresponding to the presence of crusts on the tumor surface and polygonal nucleated cells with a bright rim around a dark nucleus (parakeratosis). Hyperkeratosis and ulceration could impair the evaluation of the lesion.

Currently, the diagnostic distinction between AK and SCC, especially when solely based on clinical aspects, may not always be easily. Obtaining biopsies is an invasive method and it is not always possible to do. Peppelman et al. (Peppelman et al. 2015, 1302–9) try to assess in vivo RCM features that are specific enough to make a distinction between AK and SCC using RCM as a non-invasive in vivo diagnostic method. They demonstrated that the presence of architectural disarray in the stratum granulosum in combination with architectural disarray in the spinous layer and/or tumor nest in the dermis were the main RCM features to distinguish SCC from AK.
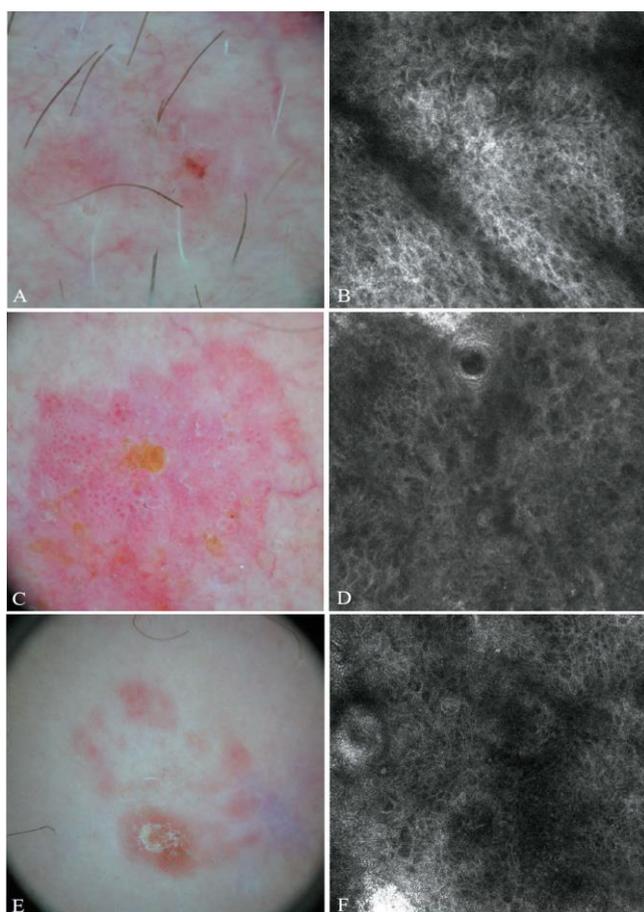
Figure 3. Dermoscopic and confocal pictures of aktinic keratosis (AKs) and squamous cell carcinoma (SCCs). A) Dermoscopic image of AK with strawberry pattern; B) confocal image of the epidermis showing a light atypical honeycombed, with the presence of irregular keratinocytes for shape and size; C) dermoscopic image of Bowel disease showing scales and glomerular vessels; D) confocal image showing mild irregular honeycombed; E) dermoscopic image of invasive squamous cell carcinoma showing scales, erosion and atypical vessels; F) confocal image showing a severe atypical honeycombed typical of SCC (Guida et al. 2015, 547–63).

From in vivo to ex vivo

Advantages related to in vivo RCM consist of real-time observation, underlying different clinical applications of RCM in the evaluation of melanocytic and non-melanocytic lesions, and in particular in the study of facial maculae and amelanotic/hypopigmented tumors and management of subclinical margins, recurrences, or monitoring noninvasive treatment of tumors (Castro et al. 2015, 1164–69; Fraga-Braghiroli et al. 2014, 933–42; Cinotti et al. 2014, 912–918.e2; García-Hernández et al. 2013, 205256).

In the last few years a new technique became available: ex vivo fluorescence confocal microscopy (FCM). FCM enables real-time imaging of skin morphology directly in freshly excised tis-

sue. For this reason, the application of interest is rapid detection of residual BCC in skin excisions during Mohs surgery (Bennàssar et al. 2014, 360–65) .

FCM requires a contrast agent that is able to highlight nucleated cells; this means that with the use of this technique only a few fluorescence is collected from the dermis while one of limit of RCM is the reflectance coming from the dermis consisting of a strong bright scattering interference.

Other perspectives are now available in this field considering that a new generation ex vivo confocal laser scanning microscopy (CLSM) device is now available. This tool enables the identification of different layers of the epidermis, differentiating keratinocytes from melanocytes and permitting the visualization in detail of skin appendages including hair follicle, sebaceous and sweat glands (Hartmann et al. 2016, 376–87).

Conclusions

RCM is a developing technology that allows optical sectioning of an area of skin without the nedd for physical sectioning: it is thus ideal for dermatologists examining detailed features of a skin lesion without troubling the patient for a biopsy specimen, for selection of the optimal site when an invasive biopsy is indicated, and for dermatological surgeons determining the margins of a lesion to be excised. The development of advanced non-invasive diagnostic techniques allows tissue imaging in vivo, and recently also ex vivo, contributing to a more accurate diagnosis of skin cancers.

While histopathology remains the gold standard for the diagnosis of skin cancers, RCM is a pivotal tool that provides clues for diagnosis of pigmented and non-pigmented lesions, flat or nodular. This technique enables non-invasive exploration of skin lesions, also in difficult-to-explore sites, identifying clues of malignancy, monitoring efficacy of topical therapies, providing a support for surgical margins and recognizing different tumor subtypes.

Disclosure

The authors declare that do not have a conflict of interest and that do not have a financial rela-

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Contributors

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

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Chronic Obstructive Pulmonary Disease (COPD) Nocturnal Desaturator patients associated with Obesity and Lung Microbiota Dynamics

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Abstract

COPD is often accompanied by other chronic diseases that are also associated with systemic inflammation, such as obesity, diabetes, and arteriosclerosis. Recent data indicate that nocturnal oxygen desaturation and coexisting metabolic syndrome are related to systemic inflammation in patients with COPD. Alveolar hypoxia and consequent hypoxemia increase in prevalence as COPD severity increases. Chronic hypoxemia contributes to the development of adverse sequelae of COPD, such as pulmonary hypertension, atherosclerosis, lung microbiome diversity and systemic inflammation. COPD is a heterogeneous disease with airway inflammation driven by bacterial infections, few studies to date they examined microbiology or infection. The introduction of culture-independent techniques for the microbiological analysis of respiratory samples confirmed that respiratory system hosts a large number of microorganisms, which include a wide range of bacteria. Regular exposure to tobacco smoke, lifestyle and food pattern can change the microbiome in healthy smokers and at-risk COPD patients, increasing the presence of a limited number of genres that reach a high relative abundance, a pattern, which can be considered as a dysbiosis. We assume that in patients with COPD and concurrent obesity and lung dysbiosis at least three factors play a role in the systemic inflammatory syndrome. Recent data showed that changes in the lung microbiome are associated with numerous exacerbations of COPD and are involved in mediating inflammatory responses of the host in some patients. Further research should elucidate the complex relationship between obstructive lung disease, obesity and lung microbiome diversity systemic inflammation accompanying these different conditions, and the causative role of systemic inflammation in obese COPD with nocturnal oxygen desaturation.

Keywords: COPD, BPCO, microbiome.

Introduction

Chronic obstructive pulmonary disease (COPD) is a disease associated with chronic inflammation of the airways and lung parenchyma. (Faner and Agustí 2017) Yet, COPD is a complex, multi-component, heterogeneous disease, whose clinical, functional presentation greatly varies from patient to patient despite similar degree of airflow limitation. (Faner and Agustí 2017; Burgel et al. 2017) The current classification of airways disorders is imprecise with an overlap of phenotypes (e.g. asthma, chronic bronchitis and emphysema), resulting in difficulties of differentiating the disorders from one another.

The prevalence, distribution and interrelationships of the main clinical and functional manifestations of the disease in a large, well-characterised and controlled population of patients are lacking. Comorbidities such as chronic heart failure, cardiovascular disease, depression, diabetes, muscle wasting, weight loss, lung cancer, and osteoporosis can frequently be found in patients with COPD and are considered part of the commonly prevalent non-pulmonary sequelae of the disease. (Barnes and

Celli 2009, 1165–85; Chatila et al. 2008, 549–55) Sleep-disordered breathing and COPD are among the most common pulmonary diseases. Nevertheless, the pathological mechanisms and clinical manifestations of COPD are not restricted only to pulmonary inflammation and airway remodelling. In contrast, over the last decade, the recognition of COPD as a systemic disease has developed. (Barnes and Celli 2009, 1165–85)

Low-grade systemic inflammation can be defined as a two- to fourfold elevation in circulating levels of proinflammatory and anti-inflammatory cytokines, naturally occurring cytokine antagonists, acute phase proteins, as well as minor increases in counts of neutrophil and natural killer cells. (Brüünsgaard and Pedersen 2003, 15–39) Systemic inflammation is considered a hallmark of COPD and one of the key mechanisms that may be responsible for the increased rate of comorbidities, including cardiovascular complications, cachexia and muscle dysfunction, osteoporosis, anaemia, clinical depression and anxiety in COPD (Sin and Man 2003, 1514–19)

The human microbiota consists of 10-100 billion symbiotic microbial cells hosted by each person, mainly bacteria in the lung; the human microbiome is made up of the genes that these cells host (Turnbaugh et al. 2007, 804–10). These cells are likely to influence genetic influences outcomes of pulmonary disease and microbiota composition through the modulation of the host's defense mechanisms.

There are few studies on genetic modifiers of exacerbation risk in COPD (Erb-Downward et al. 2011, e16384).

We assume that in patients with obese COPD and diabetes mellitus type II both genes and lifestyle are influenced. All this is known, but the genes in the human microbiome also may play an important role in the systemic inflammatory syndrome: the severity of pulmonary impairment, the degree of obesity-related adipose tissue hypoxia, and the severity of systemic hypoxia due to reduced pulmonary functions. The gut bacteria synthesize vitamins and proteins and help degrade toxins. Many genes in the human microbiome produces hormones, neurotransmitters, cytokines and molecules of inflammation, which are released into the systemic circulation and affect health.

Recent evidence demonstrated that the microbiome might affect in the COPD patients including patients with obesity and diabetes type II (Eduard Monsó 2017, 251). Exposure to tobacco smoke modifies the lung microbiome in healthy smokers, first in the oropharynx, increasing the presence of a limited number of bacteria that reach a high concentration in the lower respiratory tract, a pattern that can be considered pathological as a dysbiosis pulmonary. In the COPD, the microbiome analyzes of the sputum samples have shown an important change in bacterial diversity, with a variation in a restricted flora with an over-representation of the Proteobacteria strand, which includes most of the bacteria considered to be pathogenic microorganisms, together with a decrease of the relative abundance of microorganisms' part of the phylum Firmicute-s. Severity of COPD also influences degree of oxygen desaturation and obesity and lifestyle. Several data suggest that the pulmonary microbiome plays a fundamental role during the exacerbations of COPD and its role in the aetiology of the disease remains poorly understood.

This brief review focuses on COPD and nocturnal hypoxaemia with obesity and lung microbiome and dysbiosis that can be the cause of systemic inflammation and vascular atherosclerosis.

Systemic and airway inflammation

Several studies have found markers of systemic inflammation, such as high-sensitivity C-reactive protein (hs-CRP), to be higher in blood of patients with COPD when compared with the blood values of subjects without COPD. (Pinto-Plata et al. 2006, 23–28; Leonardo M Fabbri and Rabe 2007, 797–99). A question arises whether systemic inflammation is the result of a local inflammation spill-over to the systemic compartments or a systemic component of COPD not necessarily related to the local inflammatory processes in the lung. (L M Fabbri et al. 2008, 204–12; Broekhuizen et al. 2006, 17–22)

As a point of interest, it should be noted that systemic inflammation has failed, so far, to show substantial correlations with airway obstruction, (de Torres et al. 2006, 902–7; Watz et al. 2009, 1039–46) whereas a connection has been reported between local inflammatory pro-

cesses and airway obstruction. (Peleman et al. 1999, 839–43; Turato et al. 2002, 105–10; Baraldo et al. 2004, 308–12) Markers of systemic inflammation have been shown to be elevated in blood of patients with COPD when compared with control subjects without COPD. COPD is often accompanied by other chronic diseases that are also associated with systemic inflammation, such as chronic heart failure, diabetes, and arteriosclerosis. (Roy et al. 2009, 41) Alternatively, increased levels of inflammatory mediators in the blood of COPD patients may stem from extra pulmonary cells (circulating leukocytes, endothelium or muscle cells). A particular problem in COPD patients with marked alveolar wall destruction is intermittent and continuous hypoxia. Significant inverse correlation between arterial oxygen tension (PaO_2) and circulating tumor necrosis factor alpha (TNF-a) and soluble tumor necrosis factor receptor (sTNF-R) levels in patients with COPD has been reported. (Takabatake et al. 2000, 1179–84) Similarly, a significant relationship between reduced oxygen delivery and TNF-levels in the peripheral circulation, highlighting the role of nocturnal hypoxia of the tissue was found (Yu et al. 1998, L818-26)

It has been suggested that systemic inflammation may explain part of the heterogeneity of COPD phenotypes, such as loss of lean body mass and the higher prevalence of co morbid disorders such as coronary heart disease (CHD), depression and hypertension. Finally, this study reinforces the view that systemic inflammation is an important phenotypic feature of COPD (Eagan et al. 2010, 540–48). Future prospective studies should investigate whether these markers will give important prognostic information in relation to disease progression and severity in COPD.

Sleep disturbance in COPD

COPD alone can cause subjective and objective changes during sleep. When patients with either chronic bronchitis or emphysema were surveyed across a broad range of symptoms, "sleep difficulties" were endorsed as occurring "almost always" or "always" in 43% of subjects (third most common, after dyspnoea and fatigue). (Kinsman et al. 1983, 755–61) More than just the diagnosis of COPD, the presence of COPD symptoms such as cough or sputum production

or wheezing strongly correlated with difficulty in falling or staying asleep.(Klink, Dodge and Quan 1994, 151–54) Other investigations have objectively confirmed poor sleep quality, with decreased total sleep-time and decreased sleep efficiency. (Douglas et al. 1982, 840–44). A brief review of the normal changes in respiration that occur with sleep onset and the various sleep stages is useful to understand the changes occurring during sleep in patients with COPD. In healthy subjects, minute ventilation drops from wakefulness to non-rapid-eye-movement (non-REM) sleep, and drops further during REM sleep (about 15%,compared to the awake value) (Douglas et al. 1982, 286–89). Most of the drop in minute ventilation is due to a decrease in tidal volume not fully compensated by a concomitant increase in respiratory rate. There is a blunted ventilatory response to hypoxia and hypercapnia, again with the greatest changes during REM sleep. (Douglas et al. 1982, 758–62) Sleep-related hypoventilation has been demonstrated in COPD, particularly during REM sleep, with associated oxygen desaturation. (Hudgel et al. 1983, 669–77).

Nocturnal oxygen desaturation in COPD is likely to be the consequence of the combined effects of physiological hypoventilation during sleep. However, there is evidence that some patients with awake PaO_2 levels in the mildly hypoxaemic range can also develop clinically significant nocturnal oxygen desaturation, which may predispose to pulmonary hypertension. (Fletcher et al. 1987, 604–8)

Finally, possible mechanisms responsible for this reduction include respiratory muscle hypotonia, cephalic displacement of the diaphragm and a decrease in lung compliance. (Johnson and Remmers 1984, 1011–17).

Sleep-related breathing disturbances: nocturnal oxygen desaturation and alveolar hypoventilation.

Sleep-related hypoventilation has been demonstrated in COPD, particularly during REM, with associated oxygen desaturation (Hudgel et al. 1983, 669–77) There is a close relationship between the awake arterial oxygen tension PaO_2 and nocturnal oxygen saturation $SatO_2$ levels, (Connaughton et al. 1988, 341–44) although hypercapnia is associated with a more pronounced nocturnal oxygen desaturation than

normocapnia for any given level of waking $SatO_2$.(Bradley et al. 1990, 308–12)

Nocturnal hypoxemia has been defined as a $SatO_2$ of ≤ 90% for at least 5 min with a Nadir $SatO_2$ of ≤ 85%. Time in bed has been defined as the time from the start to the end of the recording. The percentage of total recording time (TRT) has been defined as the time spent in bed -- sleep latency + intrasleep wakefulness. The TRT spent in bed with a $SatO_2$≤ of 90% has been defined as the T_{90}%. The minimal TRT required for a satisfactory analysis of nocturnal recordings was/has been 2 hours. COPD patients with a T_{90} ≥ of 30% and a Nadir $SatO_2$ of 85% have been defined as Desaturators (D) and the others as Non-Desaturators (ND). (Toraldo et al. 2005, 3828–37) In this study the authors, as revealed by cluster analysis, have shown that clinical parameter predictors when awake from nocturnal desaturation were different. COPD D patients may be identified by a clinical pattern of variables T_{90}, Mean Pressury Artery Pulmonary (MPAP), and $PaCO_2$ values, rather than by T_{90} alone, with the latter two variables being predictors of nocturnal desaturation severity. This study has revealed the complexity of the nocturnal desaturation that many clinical variables describe not only as respiratory when awake. Alveolar hypoventilation probably accounts for most of the oxygen desaturation. Becker and colleagues (Becker et al. 1999, 112–18) measured minute ventilation during wakefulness, non-REM sleep, and REM sleep in normal subjects and patients with COPD. The greater drop in minute ventilation in subjects with COPD may reflect increased dependence on accessory muscles that become hypotonic during sleep, particularly during REM sleep.

An alternative explanation comes from the work by O' Donoghue and colleagues (O'Donoghue et al. 2004, 663–73) who have found an even greater drop in minute ventilation during non-REM sleep in hypercapnic COPD patients.

Clinical impact of nocturnal oxygen desaturation

The exact prevalence of Pulmonary Hypertension (PH) in patients with COPD is unclear. (Naeije 2005, 20–22) PH is a complication of advanced COPD observed in patients who show severe longstanding hypoxaemia. Even if PH is generally mild to moderate in most COPD patients, it may markedly worsen during acute exacerbations, sleep and exercise and these acute increases in PH could facilitate the development of right heart failure. (RHF) The diagnosis of PH in COPD patients is difficult. The published studies differ not only in their definition but also for conditions under which PH has been reported (rest, exercise, and exacerbation). According the European Society Cardiology and European Respiratory Society (Galie et al. 2009, 1219–63) PH has been defined as an increase in mean pulmonary arterial pressure PAP ≥ 25 mmHg at rest as assessed by right heart catheterisation. (RCH) The definition of PH on exercise as a Pulmonary Artery Pressure (PAP) ≥ 30 mmHg as assessed by RHC is not supported by published data and healthy individuals can reach much higher values.

The incidence of PH in COPD patients has been evaluated by Kessler and colleagues. (Kessler et al. 2001, 219–24) The authors have performed a longitudinal study on 131 patients with COPD by performing serial RHC at baseline and then at follow-up (mean follow-up was 6.8 ± 2.9 years). All subjects have had normal mean PAP at rest (≤ 20 mm Hg). They have been divided into two groups based on presence or absence of elevated mean PAP with exercise (≥30 mm Hg) 25% of patients developed PH on follow up and was mild by hemodynamic criteria (mean PAP 26.8 ± 6.6 mm Hg). Subjects who showed elevated PH with exercise were more likely to exhibit resting mean PAP elevation upon follow up. The rate of progression was +0.4 mm Hg per year.

Nocturnal oxygen desaturation seems to contribute to the development of PH; even in the absence of significant awake hypoxemias. (Fletcher et al. 1989, 757–64) REM-associated falls in $SatO_2$ are associated with increases in pulmonary artery pressure during sleep that can be reversed by supplemental oxygen, although most COPD patients with sustained pulmonary hypertension are also hypoxaemic during the daytime. Various arrhythmias are also reported

during episodes of nocturnal desaturation. (Douglas et al. 1982, 758–62) These consequences might help to explain why nocturnal oxygen desaturation is a marker of increased mortality, and why COPD patients are reported to die more frequently at night than expected (W T McNicholas and Fitzgerald 1984, 878)

Tissue hypoxia is another mechanism that can contribute to systemic inflammation in COPD. In a clinical study (Yu et al. 1998, L818-26) it has been shown that TNF-a and receptor levels were significantly higher in patients with COPD, but significantly correlated with the severity of arterial hypoxaemia. These results suggest that arterial hypoxaemia in COPD is associated with activation of the TNF-a system in vivo.

The systemic effects of inflammation may significantly contribute not only to respiratory abnormalities, symptoms and functional impairment (e.g. exercise intolerance) associated with COPD but also to its chronic marked changes of vasomotor and endothelial function as pulmonary vascular disease (Mal 2007, 114–19) The nocturnal desaturation–reoxygenation sequence is a typical pattern coupled with the majority of respiratory events. This sequence, defining intermittent hypoxia (IH), leads to oxidative stress, with production of reactive oxygen species. (ROS) (Lavie 2003, 35–51) Hypoxia induced pulmonary vasoconstriction is a protective response to keep ventilation-perfusion ratio optimum by shunting blood away from the hypoxemic areas. The traditional hypoxic model of PH is based on the hypothesis that chronic hypoxia initiates vascular remodelling leading to permanent changes in pulmonary vasculature. Studies performed in vitro elucidated the mechanisms underlying hypoxia driven vascular changes. Barbera et al., (Barberà et al. 1994, 423–29) have evaluated COPD patients undergoing lung resection and demonstrated that vascular changes contribute to vascular remodelling and reputedly may have an effect on vascular dynamics leading to PH. Nocturnal hypoxia may induce endothelial cells to release proliferate cytokine leading to cellular hypertrophy in the vessel wall and increase in extra cellular matrix. In conclusion, the nocturnal hypoxic insult occurring during sleep-disordered breathing may also contribute to chronic vascular remodelling; these mechanisms generate

vascular endothelial damage and dysfunction increasing the risk of pulmonary hypertension in COPD. (Jyothula and Safdar 2009, 351–63) The review by Mc Nicholas shows how disease act through similar pathways to cause cardiovascular disease. (Walter T McNicholas 2009, 692–700) Another intriguing possibility reported in that study is that nocturnal desaturation in COPD may contribute to an increased incidence of COPD exacerbations, which may accelerate lung-function decline and be associated with greater mortality (Donaldson et al. 2002, 847–52; Soler-Cataluña et al. 2005, 925–31).

Interaction of COPD and Obesity in Systemic Inflammation

Recently, the concept of systemic inflammation as a consequence of spill over of inflammatory mediators from the lungs to the systemic compartment in COPD has been broadly discussed. (Chung and Adcock 2008, 1334–56; van Eeden and Sin 2008, 224–38; Kim, Rogers and Criner 2008, 478–85) Several factors likely play a role in the genesis of systemic inflammation of COPD. These include tobacco use, airway inflammation, airflow obstruction, and hyperinflation. However, an independent role for tissue hypoxia seems likely. (Agusti 2005, 367–70)

The transcription factor nuclear factor $\varkappa$B (NF$\varkappa$B) is the master regulator of cellular inflammatory responses, controlling expression of key inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and interleukin-8. (Garvey, Taylor and McNicholas 2009, 1195–1205) Evidence of a role for hypoxia in the induction of an NF$\varkappa$B response comes from in vitro, in vivo, and clinical studies. Intermittent hypoxia is classically seen in patients with obstructive sleep apnea syndrome, but may arise in COPD, particularly during sleep or exertion. In sustained hypoxia, NF$\varkappa$B appears to interact with HIF-1α to promote the expression of inflammatory genes, such as cyclo-oygenase II. (Fitzpatrick et al. 2011, 1091–96)

Similarly, in a rodent model, 24 hours of sustained hypoxia has been shown to up regulate NF$\varkappa$B activity in pulmonary and cardiac tissue. (Fitzpatrick et al. 2011, 1091–96) Meanwhile, clinical studies in COPD patients have found that circulating levels of TNFα and soluble TNF receptors increase as arterial oxygen ten-

sion decreases. (Takabatake et al. 2000, 1179–84).

A number of studies have suggested it may result from "overspill" of inflammatory mediators from the lungs and pulmonary circulation, while others have failed to find any correlation between measurable pulmonary and circulating inflammatory mediators. (Sinden and Stockley 2010, 930–36) One potentially important source of inflammation in

obese patients with COPD with nocturnal hypoxaemia is white adipose tissue. In patients with COPD, obesity is characterized by an absolute abundance of fat mass (FM), similar to other diseases associated with excessive adiposity. The prevalence of obesity is the highest among patients with milder forms of the disease (GOLD Stages 1 and 2), and the lowest in patients with the most severe lung function impairment in Stage 4. (Sin and Man 2003, 1514–19; M Poulain et al. 2008, 35–41) Marquis et al. (Marquis et al., 226-32-4) demonstrated the presence of one or more components of the metabolic syndrome in almost 50% of COPD patients. High adiposity and fat tissue accumulation impair pulmonary functions and exercise performance. (Magali Poulain et al. 2006, 1293–99) Obesity and the presence of metabolic syndrome are related to increased insulin resistance in overweight and obese COPD patients (Bolton et al. 2007, 121–26)

The study by Bolton et al. (Bolton et al. 2007, 121–26) suggests that insulin resistance is aggravated by both, high BMI and increases in circulatory inflammatory mediators such as IL-6 in these patients. Indeed, inflammatory mediators TNF-α, IL-6, and leptin were significantly higher while plasma adiponectin levels were reduced in the presence of excess weight in COPD patients. Chronic low-grade adipose tissue inflammation in obesity may represent a specific response to relative hypoxia of adiposities. (Trayhurn and Wood 2004, 347–55) Several factors may contribute to cell hypoxia within adipose tissue in association with high adiposity: (a) blood flow per unit adipose tissue mass is reduced in obese humans resulting in decreased blood supply to the tissue; (b) large adipocytes are further from the vasculature than the normal diffusion distance for O_2.

Adipocyte tissue hypoxia has detrimental effects on cell metabolism and function, as evidenced by studies in

vitro and animal models. Studies in vitro have shown that hypoxia results in enhanced TNF-α production, increased expression of PAI-1, and reduced adiponectin and peroxisome proliferators-activated receptor gamma (PPARγ) expression (Chen et al. 2006, 549–56; Hosogai et al. 2007, 901–11)

On the other hand, however, obesity-related hypoxia evokes local inflammatory response within adipose tissue per se, and systemic hypoxia likely contributes to the adipose tissue inflammation. If so, elevated circulating levels of inflammation-related proteins may reflect also spill over from the adipose tissue to the systemic circulation in patients with COPD and concurrent obesity.

Even in the absence of COPD, obesity is associated with small airways dysfunction, decreased chest wall

compliance, V/Q mismatch, and increased peripheral oxygen consumption, all potentially leading to relative hypoxemia. Risk of sleep-disordered breathing and consequent nocturnal hypoxemia correlates with the degree of obesity, (Young et al. 1993, 1230–35) and in extreme cases, morbid obesity can lead to profound alveolar hypoventilation, with chronic hypercapnic respiratory failure. Dysregulated ventilatory control is another factor contributing

to the occurrence and persistence of hypoxemia in COPD patients (Kessler et al. 2001, 369–76)

The lung microbiome diversity plays an important role in COPD exacerbations: microbiota study.

The introduction of bacterial culture techniques for the microbiological analysis of respiratory samples confirmed that the respiratory system hosts many microorganisms (microbiome), including bacteria, viruses and broad-spectrum fungi. Studies using culture-independent techniques such as PCR amplification and sequencing the ribosomal RNA 16 (R) 16S gene have investigated a distinct bacterial community in the airways of COPD patients compared to healthy subjects and suggest that changes in the microbiota pulmonary disease could be associated with inflammation and airway exacerbations and disease progression. However, most

studies on pulmonary microbiome involved small groups of subjects with limited longitudinal sampling and modest clinical information. (Huang et al. 2014, 2813–23; Zakharkina et al. 2013, e68302) Acute exacerbations of COPD are a rapid worsening of symptoms in which the role of bacteria is one of the major etiological factors (E Monsó et al. 1995, 1316–20; Soler et al. 1998, 1498–1505) Then, the dynamics of bacterial prevalence during exacerbations and its role in the pathogenesis of the disease remain poorly known.

Most of the studies on COPD lung microbiota have used sputum as a representative sample of the respiratory system, thanks to its easy recovery and standardized processing procedure. In the respiratory microbiome analysis, it should be considered that the sputum comes mainly from the proximal airways, which possess a flora that showed clear differences with the microbial pattern found in the distal bronchi and in the alveolar space (Cabrera-Rubio et al. 2012, 3562–68; Pragman et al. 2012, e47305) which is studied on the contrary through bronchial biopsies, and / or bronchoalveolar lavage, while in COPD the microbial diversity model has been shown to be different between the microbiological examination of the sputum (upper airways) and the microbiological examination coming from the distal samples (lower airways), a finding that confirms the differences between proximal and distal bronchial flora. Microbial cultures have correlated respiratory symptoms in COPD exacerbations to the detection of new strains in bronchial flora (Sethi and Murphy 2008, 2355–65) but this change in bacterial flora does not occur in all disease exacerbations. The microbiome analyzes show a high relative abundance of specific bacterial genera, which can be considered etiological, for most exacerbations, while the remaining bacterial flora does not change significantly (Millares et al. 2014, 1101–11; Dy and Sethi 2016, 196–202) Furthermore, exacerbations are not only related to the prevalence of isolated bacterial genes, but are also associated with changes in the composition of the microbiome as a whole, but not always identifiable through the measurement of the relative infectious microbial load (Molyneaux et al. 2013, 1224–31).

An increase in the relative abundance of a specific bacterial genus can be considered causal in a COPD, but often subsequently, it is not confirmed by culture results. Several studies have showed that bacteria prevalent in the analysis of bronchial secretions may not be confirmed by the examination of the culture, while the microorganisms of the culture grow easily from the sample analysed, although their relative abundance has not changed compared to the previous stability (Sethi et al. 2006, 991–98; Millares et al. 2014, 1101–11) The microbiome analyses demonstrated a different pattern in infectious and eosinophilic exacerbations, with a clear prevalence of the Firmicutes genus in eosinophilic exacerbation, in the face of the predominance of Proteobacteria in exacerbations that show positive cultures for bacteria (Wang et al. 2016, 1082–92) This finding supports the clinical characterization of exacerbations in these two categories (bacterial and eosinophilic), considering that their microbial pattern is completely different and will require different therapeutic approaches. The interaction between different bacterial microorganisms can be addressed through analysis of microbiomes and in a clinical model; an important role of viral infection on the composition of the microbiome in patients with COPD has been demonstrated. Induced rhinovirus infection showed no effect on the microbiome of bronchial secretions in healthy subjects, but was associated with an increase in the prevalence of Proteobacteria in patients with COPD (Molyneaux et al. 2013, 1224–31). This observation confirms the role of rhinovirus infection as an inducer of changes in respiratory bacterial flora with prevalence of Proteobacteria and justifies the virus-virus detection of coinfections in one quarter of COPD exacerbations (Papi et al. 2006, 1114–21) The presence of bacterial diversity, often related to a relative increase in the bacterium Proteobacteria, is associated with greater severity in COPD and may be one of the determinants influencing the progression of exacerbations and pulmonary disease, as previously demonstrated in idiopathic pulmonary fibrosis (Molyneaux and Maher 2013, 376–81).

Conclusion

The hypoxic insult occurring during sleep-disordered breathing to COPD varies from one condition to another. However, there are com-

mon cardiovascular and metabolic morbidities in these various conditions

There are major differences with continuous hypoxia, suggesting specific pathways originating from the occurrence of oxidative stress and inflammatory cascade activation. Nocturnal hypoxaemia during sleep and diurnal hypoxaemia seems to be the major factor in morbidity. Also, the hypothesis that adipose tissue may contribute to the overall systemic inflammatory phenotype in patients with early stages COPD with obesity or relative abundant fat mass is novel (Millares et al. 2014, 1101–11). The recent role played by the lung microbiota in systemic inflammation is been presented. The microbial composition of the lung bacterial flora in COPD has been studied through microbiome analysis, the involvement of respiratory flora in the pathogenesis of the disease, it is practically unknow. In conclusion, recent data showed that lung microbiota is dynamic in which rapid changes appeared to be associated with exacerbations and disease progression events indicative of specific exacerbation phenotypes.

Disclosure

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

Contributors

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

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Vaginal infections in diabetic woman

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Abstract
Diabetes mellitus is a chronic disease that can affect any organ or system of the body. Diabetes mellitus has long been considered as one of the factors causing Candida vaginitis and has the potential to affect sexual function in women. Infectious diseases are more prevalent in individuals with Diabetes Mellitus. One of the problems of women with diabetes is resistant vulvovaginitis, which is related to some factors such as neuropathy, hyperglycemia, allergy and atopy. Women's interest in, satisfaction with, and ability to participate in sexual activity may be influenced globally by the effect of diabetes on their overall health, physical and mental functioning, and interpersonal relationships. Additionally, sexual function may be adversely affected by diabetes medications or other health interventions. This manuscript is a comprehensive review on vulvovaginal infections in diabetic patients.

Keywords: Diabetes, vulvovaginal infections, vaginitis, Candidiasis, recurrent infections, genital yeast infections, genital mycotic infections, fungal infections, sexual dysfunction.

Introduction

Diabetes is a chronic disease, which occurs when the pancreas does not produce enough insulin, or when the body cannot effectively use the produced insulin. This leads to an increased concentration of glucose in the blood (hyperglycemia). Type 1 diabetes (previously known as insulin-dependent or childhood-onset diabetes) is characterized by a lack of insulin production. Type 2 diabetes (formerly called non-insulin-dependent or adult-onset diabetes) is caused by the body's ineffective use of insulin. It often results from excess body weight and physical inactivity. Gestational diabetes is hyperglycemia that is first recognized during pregnancy. 346 million people worldwide have diabetes. In 2004, an estimated 3.4 million people died from consequences of high blood sugar. WHO projects that diabetes death will double between 2005 and 2030 (WHO, 2011)**.** Diabetes is characterized by elevated morbidity and mortality. Diabetes represents an economic burden, with serious implications for the public health systems. Accordingly, the need of effective tools aimed to the prevention of its chronic complications is urgent and cannot be deferred (Lavery et al. 1998, 157–62).

Besides the classical complications of the disease, DM has been associated with reduced response of T cells, neutrophil function, and disorders of humoral immunity (Geerlings and Hoepelman 1999, 259–65; Peleg et al. 2007, 3–13). Consequently, DM increases the susceptibility to infections, both the most common

ones as well as those that almost always affect only people with DM (e.g. rhino cerebral mucormycosis) (Peleg et al. 2007, 3–13). Such infections, in addition to the repercussions associated with its infectivity, may trigger DM complications such as hypoglycemia and ketoacidosis. Infectious diseases are more prevalent in individuals with DM. The main pathogenic mechanisms are hyperglycemic environment increasing the virulence of some pathogens; lower production of interleukins in response to infection; reduced chemotaxis and phagocytic activity, immobilization of polymorphonuclear leukocytes; glycosuria, gastrointestinal and urinary dysmotility. Some infections usually affect only diabetic persons, such as malignant external otitis, rhino cerebral mucormycosis, and gangrenous cholecystitis. In addition to being potentially more serious, infectious diseases in DM may result in metabolic complications such as hypoglycemia, ketoacidosis, and coma (Casqueiro, Casqueiro and Alves 2012, S27-36).

Diabetes causes short-term and long-term complications and usually its long-term complications develop 5 to 10 years after the diagnosis of both types of the disease (Smeltzer, S. Bare 2000). One of its long-term complications is a neurological complication that includes a disorder of sexual functioning. The autonomic system causes a wide range of disorders in all systems of our body including the urinary-sexual system. It can be said that sexual dysfunction develops frequently as a complication among these patients (Chu and Edelman 2002, 60–66). Neuropathy, vascular impairment, and psychological complications are involved in decreased libido, low arousal, decreased vaginal lubrication, orgasmic dysfunction, and dyspareunia among women with diabetes.

Moreover, one of the problems of women with diabetes is resistant vulvovaginitis, which is related to some factors such as hyperglycemia, allergy and atopy. One of the most common pathogens associated with this condition is Candida spp. (Malazy et al. 2007, 399–404).

Material and methods

The following sources were searched for the original study: The Cochrane Library, MEDLINE, EMBASE and the Cochrane sexually transmitted disease (2000-2014). Selection criteria were:

- ▪ Randomized controlled trials published in any language;
- ▪ Women (age 16 years or over) with uncomplicated vulvovaginal candidiasis;
- ▪ The vulvovaginal candidiasis to be made mycological (i.e. positive culture and/ or microscopy for yeast);
- ▪ The primary outcome measure was clinical cure.

Data collections and analysis

Two reviewers screened titles and abstract of the electronic search results and full text of potentially relevant papers. Two reviewers performed independent duplicate abstraction. Disagreements regarding trial inclusion or data abstraction were resolved by discussion between the reviewers. Odds ratios were pooled using the fixed effects models (except for two analyses when random effects models were used because of potentially important heterogeneity).

Normal vaginal flora

Anatomically, the female genital tract is constituted by a succession of cavities that communicate with exterior through the vulvar cleft. Vaginal microflora undoubtedly presents one of the most of important defense mechanism for the reproductive function, maintaining the environment healthy and preventing the proliferation of microorganisms, stranger to the vagina (Linhares, Giraldo and Baracat, 370–74). The different components of the vaginal ecosystem have been observed on the microscope and afterwards, identified through culture-specific techniques (Larsen and Monif 2001, e69-77). However, more recently, identification techniques for independent bacteria in the means of culture have revolutionized the study of microorganisms. The use of amplification, cloning techniques and subsequent analysis of sequences of bacterial genes (genes that codify for bacterial rRNA 16S) in sample of vaginal fluid have allowed the identification of the majority of common species of Lactobacilli and other microorganisms. Thus, these techniques have demonstrated that Lactobacilli spp. do not always correspond to the dominant species in the vagina of healthy women. Besides that, vagina environment inhabitants until then unknown have been identified (Zhou et al. 2004, 2565–73; Fredricks, Fiedler and Marrazzo 2005,

1899–1911). Although vaginal bacterial species of healthy women have been initially identified as Lactobacillus acidophilus, this knowledge represents a simplification, since in women whose flora is dominated by Lactobacillus, the more frequently detected species through genic amplification are L. crispatus and L. inners (Zhou et al. 2004, 2565–73; Fredricks, Fiedler and Marrazzo 2005, 1899–1911) or L. crispatus and L.gasseri (Verhelst et al. 2004, 16). Other species as. L. jensenii, L. gallinarum and L. vaginalis have also been identified in some women. A study on vaginal flora accomplished in three continents using method of bacterial genes analysis showed that dominant species were the same in each region: L. crispatus, L.gasseri and L. jensenii (Pavlova et al. 2002, 451–59) Besides, that, the same study observed that, in some women, the normal ecosystem was maintained in the absence of Lactobacillus; in one woman Atopobium vaginae was identified as the dominant microorganism in the flora and, in two other woman, the bacteria Atopobium, Megasphaera and Leptotrichia were all lactic acidic producers similarly Lactobacillus. (Zhou et al. 2004, 2565–73; Rodriguez Jovita et al. 1999, 1573–76). Therefore, other bacteria, not only by Lactobacillus, may keep the vagina's acidic environment, known as an important defense mechanism against the proliferation of pathogens. Characteristic of vaginal microbial flora is the presence of biofilms. Biofilms are formed by colonies of microorganisms that adhere among themselves and cover a solid surface. Biofilms have already been identified in the surface of vaginal cells, more known in women with bacterial vaginosis, where species of Gardnerella vaginalis and Atopobium predominate (Swidsinski et al. 2005, 1013–23). The occurrence of species of Lactobacilli producers of hydrogen peroxide, which possess a defense activity against pathogens, seems to be less frequent in African-American women (Antonio, Hawes and Hillier 1999, 1950–56). Studies suggest that for bacterial vaginosis-free women, the vaginal pH is higher in African-American women (Stevens-Simon et al., 168–72) they suggest also that such differences are valid only for women whose vaginal microflora is not dominated by Lactobacillus (Royce et al. 1999, 96–102; Fiscella and Klebanoff 2004, 747–50). The composition of the vaginal flora is not constant, suffering variations in response to exogenous and endogenous factors (Priestley et al. 1997, 23–28; David A. Eschenbach et al. 2001, 913–18). These factors include the different phase of menstrual cycle, gestation, use of contraceptives, frequency of sexual intercourse, use of showers or deodorant products, use of antibiotics or other medications with immune-suppressive properties. Alterations occurring in the vaginal environment may increase or decrease the selective advantages for specific microorganisms. For example, studies have related the loss of Lactobacillus to sexual intercourse or the use of antibiotics (Schwebke, Richey and Weiss2 1999, 1632–36).

Nevertheless, another study has demonstrated that the sexual act without the use of condom had o effects upon the Lactobacillus, but increase the level of Escherichia coli and facultative gram-negative bacilli (D A Eschenbach et al. 2000, 901–7).

During the menstrual cycle, hormonal variations interfere in the substrate of different microorganisms; these variations lead to change s in vaginal ph. Yet the levels of Lactobacillus remain constant throughout the cycle, the non-Lactobacillus bacteria increase during the proliferative phase and the concentrations of Candida albicans become higher in the premenstrual period (Witkin 1987, 34–37).

Candida albicans is tolerant to the acidic environment, fund in approximately 10% to 20% of women in reproductive age. The concentration of the microorganism is low, so the woman carrier is asymptomatic. Nevertheless, event leading to a state of local immune-suppression, such as sexual intercourse or local induction of allergic response, create adequate conditions for the proliferation of the microorganism and facilitate the transformation into the shape of hyphae, more invasive (Witkin 1987, 34–37) Furthermore, Candida albicans appears to be able to raise the pH from 4 to > 7, resulting in auto induction of yeast-hyphal transition as demonstrated in study of Vylkova et al. (Vylkova et al. 2011, e00055-11). When the hyphal form is predominant, it results in the emergence of symptomatic vaginitis. The production of lactic acid may be essential for the maintenance of healthy ecosystem, regardless of the bacterial species that may be present in the vagina. Resulting acidic pH prevents the excessive prolif-

eration of potentially pathogenic microorganisms. It is important yet to remember that the Lactobacillus dominance is beneficial for the host, since some species produce hydrogen peroxide and bacteriocins, factors that inhibit the proliferation of the other microorganisms. In addition to the protective effects of the endogenous vaginal flora, the protection against potentially pathogenic microorganisms is done also by the local components of innate immunity and acquired immunity. The epithelial cells layer of the vagina constitutes the initial contact point between microorganism and host's genital tract. These epithelial cells possess toll-like receptors (TRL) in their surface and, therefore, are important components of innate vaginal immunity (Quayle, 61–79). Vaginal cells also release molecules with potent non-specific antimicrobial activity. A class of these molecules, known as defensives, include positively charged peptides that rapidly bind negatively charged bacterial surface (Linhares, Giraldo and Baracat, 370–74).

Vulvovaginal diseases in diabetic woman
A. Candidiasis

One of the problems of women with diabetes is resistant vulvovaginitis, which is related to some factors such as hyperglycemia, allergy and atopy. One of the most common pathogens associated with this condition is Candida albicans. Thus, most physicians begin antifungal therapy at the patient's first visit, even without paraclinical findings. The prevalence of Candida spp. vulvovaginitis and factors that cause diabetic women to be prone to this infection were analyzed in a study of B. 160 women with diabetes mellitus (mean age, 51 - 10 years; age range, 16–75 years) were enrolled. Ninety-four percent of the patients had type II diabetes mellitus and the remaining had type I. Seventy-five percent of the participants received oral hypoglycemic drugs, 15% received insulin and the rest were given combination therapy (tablets plus insulin). The mean duration of diabetes mellitus in these patients was 9 - 6 years (range, 0.08–36 years). The subjects were mainly homemakers (92.5%), and the rest were workers. There was sexual activity in 86.3% of the cases, and none in 13.7% of the cases. Twenty-one percent of our patients had a history of allergy (rhinitis, sinusitis or atopic dermatitis) (Malazy et al. 2007, 399–404).

In this study, all subjects were asked to fill out prepared questionnaires on demographic characteristics, type of diabetes mellitus, duration and type of its treatment, past history of allergy, methods of washing underwear (detergents, heat, etc.) and voiding after sexual intercourse, and vaginal examination was done for each participant by a general physician. In addition, concentrations of fasting blood sugar, 2-hour postprandial blood sugar and glycosylated hemoglobin were measured, and cultures of vaginal discharge were performed.

71% (113/160) of the women had clinical vaginitis (fungal or bacterial), and 12.5% (20/160) had Candida vaginitis. Microscopic findings for Candida were positive in 12 patients, of whom two had Candida vaginitis.

The prevalence of Candida vaginitis together with positive culture was 2.6% (4/160). After including the 10 patients with positive Candida culture together with other clinical vaginitis, the overall prevalence of Candida vaginitis based on positive culture was 8.8% (14/160). There was a significant statistical difference between either mean fasting blood sugar or educational level and infectious vaginal culture. There were no significant associations between positive culture and age, glycosylated hemoglobin, history of allergy, genital hygiene, and occupation, kind of treatment, and type or duration of diabetes.

One of the complications experienced by diabetic patients is resistant and recurrent infections. Some believe that malfunctioning leucocytes, especially in the presence of uncontrolled blood glucose levels (Wilson and Reeves 1986, 478–84; Wilson, Tomlinson and Reeves, 37–40; Raith, Csató and Dobozy 1983, 557–64) cause the condition. Among the infections, vaginal inflammation or infection, especially fungal vaginitis, is more disturbing in severe hyperglycemic conditions (Foster 1998, 2060–81) The most common etiologic agent for this infection is the yeast (fungal) organism, usually Candida (Curry 1994, 689–700; Eckert et al. 1998, 757–65; Smith 1998, 557–58; Moraes 1998, 165–69; Bornstein et al. 2001, 105–11). In a study by Eckert et al (Eckert et al. 1998, 757–65) Candida was detected in 28% of cultures of vaginal discharge obtained during the initial visit of women with vaginitis; the remaining cultures

were for other organisms, such as sexually transmitted organisms. In this case, the authors believed that the causes of recurrent disease or resistance to therapy were false diagnosis of the pathogenic organism and unsuitable treatment (Eckert et al. 1998, 757–65). On the other hand, even when correct diagnosis had been made, background factors, especially those associated with systemic diseases like diabetes mellitus, caused treatment failure (Foster 1998, 2060–81; Smith 1998, 557–58). Researchers have stressed the importance of personal hygiene, history of infections and allergic diseases of the upper respiratory tract (nose, sinuses), and atopic dermatitis in the subject or the relative(s) in recurrent diseases or resistance to therapy (Moraes 1998, 165–69).

In this study, the authors stated that there are no significant statistical differences or relationship between positive vaginal Candida culture and type of diabetes mellitus, age, and glycosylated hemoglobin, duration of diabetes mellitus, history of allergy, occupation, genital hygiene and vaginal intercourse. Diabetes mellitus has long been considered as one of the factors causing Candida vaginitis (J D Sobel 1993, 153–65; Scudamore, Tooley and Allcorn 1992, 260–63). Different studies showed that symptomatic infection is more common in women with diabetes than in the normal population (J D Sobel 1993, 153–65; Scudamore, Tooley and Allcorn 1992, 260–63). The prevalence ranged from around 7% to more than 50% (Bohannon 1998, 451–56; Sonck and Somersalo 1963, 846–52; Davis 1969, 40–45) and most of which was attributed to Candida albicans (Duerr et al. 1997, 252–56; Otero et al. 1998, 526–30).

Two specialized clinics reported that the prevalence of vulvovaginitis caused by non-albicans species is around 10–20%, with Candida glabrata dominating (Jack D. Sobel 1997, 1896–1903; Spinillo et al. 1992, 343–47). Sobel et al (J D Sobel et al. 1998, 203–11) stated that the probable causes of higher non-albicans species: the short duration of use for oral or local anti-Candida regimens; widespread use of over-the-counter antifungal agents, most of which are used incorrectly or inadequately; and the prolonged use of antifungal compounds for the prevention of recurrent vulvovaginitis, which further intensifies the problem.

The control of blood glucose levels and a suitable antifungal therapy play an important role in controlling vaginal Candida spp. infection in diabetic women (Bohannon 1998, 451–56). In a study of 241 diabetic women, a significant statistical relationship existed between overall prevalence of vaginal infections (bacterial and fungal) and mean blood glucose level. In addition, it was suggested that glucose levels be maintained below 200 mg/dL to avoid dehydration, caloric loss and glycosuria and to reduce the risk of infection. The authors also found a significant statistical difference between mean fasting blood sugar and infectious culture of vaginal discharge (p = 0.016). It did not find a significant statistical difference between glycosylated hemoglobin and infectious vaginitis, because acute infections such as vaginitis often occur during the hyperglycemic state, but glycosylated hemoglobin reflects the mean blood glucose level over the previous 3 months.

Similar to the study by Mas Martin (R Goswami et al. 2000, 162–66) B also found that most of the patients with positive vaginal Candida culture (64%) were older than 45 years. Regarding the high prevalence of Candida vaginitis in reproductive ages (Spinillo et al. 1992, 343–47; Deepti Goswami et al. 2006, 111–17) and our lack of knowledge about the prevalence of this condition in non-diabetic women with similar age groups (16–75 years), it is suggested that further evaluation be conducted to determine the cause. In any case, the incidence of symptomatic infection

is high among reproductive ages (18–44 years) (de Leon et al. 2002, 1). Although it was reported that allergic rhinitis and recurrent Candida vaginitis were present concurrently in 70% of patients (Moraes 1998, 165–69) we did not find any relationship between Candida or non-Candida vaginitis and history of allergy (sinusitis, rhinitis or atopic dermatitis). According to our results, there was significant statistical difference between educational level and infectious or positive vaginal Candida culture.

Other factors involved in the pathogenesis of Candida vulvovaginitis include lifestyle factors. Although vulvovaginal candidiasis is more frequent among sexually active women (during the second decade of life) (de Leon et al. 2002, 1) it is not considered as a sexually transmitted disease (Jack D. Sobel 1997, 1896–1903) Candida

accounts for 20–50% of the normal vaginal flora of asymptomatic healthy women (Geiger, Foxman and Sobel 1995, 304–7) and Candida vaginitis can even occur in single women. Naturally, Candida is transmitted through vaginal sexual intercourse, as well as other modes of sexual contact.

There is controversy about the frequency of sexual intercourse as a risk factor of vaginitis. However, some studies showed that oral sex increases the incidence of vulvovaginal candidiasis and that vaginal intercourse alone cannot change the Candida colonization rates in the vagina (Malazy et al. 2007, 399–404).

Yeast development can be stimulated by glucose and even be promoted to change to a more virulent stage. Pregnancy and / or diabetes increase(s) the adherence of C. albicans to vaginal epithelial cells in vitro (Grigoriou et al. 2006, 121–25).

Women with type I diabetes had higher Candida colonization rates than those with type II, even after adjusting for age, behavioral factors and HbA1c.10 Whether this is due to the diabetes type or reflects the different distributions of Candida species by age or both remains unclear. With the oxidative killing ability of neutrophils hindered, diabetics may not be able to clear pathogens as efficiently as non-diabetics may. Hyperglycemic individuals may have increased risk for Candida colonization as their secretions contain glucose, which can serve as nutrients for Candida species. A fucose (6-deoxy-galactose) vaginal epithelial cell receptor that aids in adhesion of Candida to vaginal epithelial cells was reported.30 Fucose acts as one form of the receptor site for Candida adhesion; therefore, increased Candida colonization may be proportional to the glucose level. However, glycaemia does not fully explain the observed increased risk of Candida colonization. Grigoriou et al (Malazy et al. 2007, 399–404) found an increased prevalence of vaginal candidiasis in diabetic patients compared with non-diabetic patients. Likewise, C. albicans was isolated significantly more in diabetic patients than non-C. Albicans species (Yildirim, Kilic and Kalkanci 2011, e463-7).

Large proportion of vulvovaginal candidiasis (VVC) in diabetes is due to non–albicans Candida species such as C. glabrata and C. tropicalis. Observational studies indicate that diabetic patients with C. glabrata VVC respond poorly to azole drugs. It is evaluated the response to oral fluconazole and boric acidic vaginal suppositories in diabetic patients with VVC.

112 consecutive diabetic patients with VVC were block randomized to receive either single-dose oral 150-mg fluconazole or boric acidic vaginal suppositories (600 mg/day for 14 days). The primary efficacy outcome was the mycological cure in patients with C. glabrata VVC in the two treatment arms. The secondary outcomes were the mycological cure in C. albicans VVC, overall mycological cure irrespective of the type of Candida species, frequencies of yeast on direct microscopy, and clinical symptoms and signs of VVC on the 15th day of treatment. Intention-to treat (ITT; n = 111) and per protocol (PP; n = 99) analyses were performed.

C. glabrata was isolated in 68 (61.3%) and C. albicans in 32 (28.8%) of 111 subjects. Patients with C. glabrata VVC showed higher mycological cure with boric acidic compared with fluconazole in the ITT (21 of 33, 63.6% vs. 10 of 35, 28.6%; P = 0.01) and PP analyses (21 of 29, 72.4% vs. 10 of 30, 33.3%; P = 0.01). The secondary efficacy outcomes were not significantly different in the two treatment arms in the ITT and PP analyses.

Diabetic women with C. glabrata VVC show higher mycological cure with boric acidic vaginal suppositories given for 14 days in comparison with single-dose oral 150-mg fluconazole.

The 59.9% prevalence of C. glabrata infection observed in the current study confirms the findings of our earlier studies that non-albicans VVC is frequent in diabetic women (R Goswami et al. 2000, 162–66; Deepti Goswami et al. 2006, 111–17). De Leon et al (de Leon et al. 2002, 1) observed the 54% vaginal carriage rate of C. glabrata in type 2 diabetes. The comparable frequency of clinical symptoms and signs between diabetic women with C. glabrata or C. albicans infection is similar to that reported by Geiger et al (Geiger, Foxman and Sobel 1995, 304–7) in nondiabetic women.

Increased prevalence of C. glabrata infection in diabetic women has clinical relevance because poor therapeutic response and innate resistance to azoles has been reported for C. glabrata VVC in nondiabetic women (Vermitsky and Edlind 2004, 3773–81) Similar information is

lacking in diabetic subjects, as they are often excluded in antifungal efficacy studies (Deepti Goswami et al. 2006, 111–17) Poor mycological cure in diabetic women with C. glabrata VVC to single-dose oral 150-mg fluconazole, observed in the current study, is in accordance with our earlier case-control study (Deepti Goswami et al. 2006, 111–17). In the current study, the higher mycological cure (72.4%) to boric acidic therapy in diabetics with C. glabrata VVC is similar to that reported in nondiabetic individuals.

Boric acidic or boracic [B (OH) $_3$] is a weak acidic, and its mode of antifungal action is not clear. Shiohara and Tasker (15) proposed that its acidic properties lead to disruption of the fungal cell wall. The cause of increased C. glabrata isolation in diabetic women is not clear but may involve frequent use of antifungal drugs leading to its reduced susceptibility to azoles (Ruhnke 2006, 495–504) and consequent polarization/homing in diabetic women. Feng et al (Feng et al. 2005, 445–50) reported lesser susceptibility of C. glabrata in comparison with Albicans to β-defensins, natural cationic antimicrobial/antifungal peptides expressed in human epithelia. In diabetic milieu, β-defensins expression is reduced (Froy et al. 2007, 796–802). Reduced expression of defensins in association with resistance of C. glabrata to fungicidal activity of drugs like fluconazole may also explain the high prevalence of C. glabrata VVC in diabetic women. Boric acidic therapy could be considered as the frontline therapy for treating VVC in diabetic women because it is effective against both C. albicans and C. glabrata compared with fluconazole, which is effective against C. albicans only (Ray et al. 2007, 312–17).

Sertaconazole nitrate is a new topical broad-spectrum antifungal that was developed to provide an additional agent for the treatment of superficial cutaneous and mucosal infections. Sertaconazole has been tested in vitro against clinical and laboratory isolates of the most common fungi present in superficial tinea and Candida infections. Sertaconazole is effective against a broad spectrum of organisms that cause superficial cutaneous fungal infections.

Clinical trials with Sertaconazole nitrate cream 2% show efficacy in the treatment of superficial cutaneous fungal infections (Zsolt I. 2002, 29-32). In European clinical studies of other dermatomycoses caused by Candida spp., Sertaconazole was shown to be superior to other azoles and terbinafine.

Sertaconazole nitrate has been tested with a variety of in vitro methods, all of which show fungistatic activity against dermatophytes and fungicidal and fungistatic activity against yeasts (Carrillo-Muñoz et al. 2003, 248–51).

In a similar study, Day (Grossman and Day 2005, P128) showed that over a 48-h exposure period, approximately 38% of the applied dose, or 800 Ag, penetrated the skin. These results are considerably higher than the concentrations (MICs) required in susceptibility studies to eradicate fungal growth in vitro. Thus, a short course of treatment with Sertaconazole for 4 weeks will give ratios of Cmax/MIC N1000 for dermatophytes and Candida. This level of exposure should be sufficient to obtain successful mycological cure rates of the major dermatophytes and yeas that cause superficial fungal infections; however, at present, there is insufficient data relating clinical outcome to drug exposure and MIC to allow us to assign a predictive value to these parameters. In immunocompetent patients, the majority of uncomplicated, superficial mycoses are yeast and tinea infections, caused mainly by Candida spp. and dermatophytes, respectively (Pfaller and Sutton 2006, 147–52).

The most common vulvovaginal symptom especially during candidiasis include irritation, burning, erythema and sometimes dyspareunia (Lewis et al. 2010, 1598–1607). In particular itching is reported as the more discomfort symptom (Nowosielski and Skrzypulec-Plinta 2011, 2532–45).

B. Sexual / Libido diseases

Diabetic women often deal with low sexual desire, orgasmic dysfunction, dyspareunia, and sexual aversion along with decreased lubrication often related to neuropathy.

Nerve problems can occur in every organ system, including the digestive tract, heart, and sex organs.

About 60 to 70 percent of people with diabetes have some form of neuropathy. People with diabetes

can develop nerve problems at any time, but risk rises with age and longer duration of diabetes. The

highest rates of neuropathy are among people who have had diabetes for at least 25 years.

Hyperglycemia and oxidative stress also contribute to the abnormal glycation of nerve cell proteins and the inappropriate activation of protein kinase C, resulting in further nerve dysfunction and ischemia.

However, more recent large epidemiological studies conducted on women in the United States, Brazil, and Australia did not find a significant relationship between diabetes, desire, arousal, or orgasm. In diabetic women, hypertension or the use of hypertensive drugs seem to be associated with orgasm and lubrication dysfunction as well as decreased sexual interest. Stress urinary incontinence is negatively associated with all aspects of women's sexual dysfunction (sexual interest, desire, arousal, lubrication, and orgasm), and is positively correlated with dyspareunia and vaginismus (Lewis et al. 2010, 1598–1607).

Sexual function was examined in a cross-sectional cohort of ethnically diverse women aged 40 to 80 years using self-administered questionnaires. Multivariable regression models compared self-reported sexual desire, frequency of sexual activity, overall sexual satisfaction, and specific sexual problems (difficulty with lubrication, arousal, orgasm, or pain) among insulin-treated diabetic, noninsulin-treated diabetic and nondiabetic women. Additional models assessed relationships between diabetic end-organ complications (heart disease, stroke, renal dysfunction, and peripheral neuropathy) and sexual function.

Among the 2,270 participants, mean SD age was 55 ± 9.2 years, 1,006 (44.4%) were non-Latina white, 486 (21.4%) had diabetes, and 139 (6.1%) were taking insulin. Compared to 19.3% of non-diabetic women, 34.9% of insulin-treated diabetic women (adjusted OR [95%CI] =2.04[1.32–3.15] and 26.0% of non-insulin-treated diabetic women (adjusted OR [95%CI] =1.42[1.03–1.94]) reported low overall sexual satisfaction. Among sexually active women, insulin-treated diabetic women were more likely to report problems with lubrication (OR [95%CI] =2.37[1.35–4.16]) and orgasm (OR [95%CI] =1.80[1.01–3.20]) than nondiabetic

women. Among all diabetic women, end-organ complications such as heart disease, stroke, renal dysfunction, and peripheral neuropathy were associated with decreased sexual function in at least one domain. Compared to nondiabetic women, diabetic women are more likely to report low overall sexual satisfaction. Insulin-treated diabetic women also appear at higher risk for problems such as difficulty with lubrication and orgasm. Prevention of end-organ complications may be important in preserving sexual activity and function in diabetic women.

For the purposes of analysis, participants were categorized into one of three-diabetes status groups based on whether they had diabetes, and, if so, whether they were using insulin: (1) insulin-treated diabetic women, (2) non-insulin-treated diabetic women, and (3) non-diabetic women. These categories were chosen a priori based on the recognition that insulin use is a widely recognized indicator of diabetes severity and represents a higher level of disease management burden that can interfere with day-to-day functioning and quality of life.

Differences in the demographic and clinical characteristics of participants in these three categories were examined using chi-square tests for categorical variables and analysis of covariance for continuous variables. Next, it is described the distribution of less than monthly sexual activity, less than moderate sexual desire, and less than moderate sexual satisfaction among women in each diabetes status category. Among women reporting at least some sexual activity in the past 3 months, the prevalence of specific sexual problems such as low or very low arousal, at least moderate difficulty with lubrication, at least moderate difficulty with orgasm, or at least moderate pain with vaginal intercourse were also examined among women in each diabetes status category. Differences in the distribution of these sexual function outcomes among women in different diabetes status categories were examined using chi-square tests.

Multivariable logistic regression models compared sexual function outcomes among: (1) insulin-treated diabetic versus nondiabetic women, and (2) noninsulin-treated diabetic versus nondiabetic women, adjusting for a core set of other factors with potential to influence sexual function (i.e., age, race/ethnicity, marital/relationship status, menopausal status, his-

tory of sex with men or women, body mass index, hysterectomy and oophorectomy, selective serotonin reuptake inhibitor [SSRI] use, and estrogen use.) While models examining frequency of sexual activity, desire, and satisfaction included all women, models examining specific sexual problems were confined to sexually active women, and additionally controlled for frequency of sexual activity.

Sexual activity, desire, and satisfaction outcomes were examined in all diabetic participants, while specific problems with lubrication, arousal, orgasm, or pain were examined in sexually active diabetic women only.

Of the 2,270 participants, 139 (6.1%) were insulin-treated diabetic, 347 (15.3%) were noninsulin-treated diabetic, and 1,784 (78.6%) were non-diabetic women. Mean ($\pm$ SD) age was 55 ($\pm$ 9.2) years, 1,006 (44.4%) were non-Latina white, 443 (19.5%) were African-American, 401 (17.7%) were Latina, 401 (17.7%) were Asian, and 18 (0.8%) were Native American. Overall, 63.7% of participants reported some sexual activity in the past 3 months. Of the 807 women who reported no sexual activity in the past 3 months, 271 (33.6%) indicated that lack of a partner and 224 (27.7%) indicated that partner health problems contributed to their sexual inactivity.

Insulin-treated diabetic women were less likely to report at least monthly sexual activity compared to either non-insulin-treated diabetic women or non-diabetic women. Insulin-treated diabetic women were also more likely to report low sexual desire and satisfaction compared to non-insulin-treated diabetic women or non-diabetic women (Nowosielski and Skrzypulec-Plinta 2011, 2532–45).

Among sexually active participants, problems with lubrication were also more common in insulin-treated diabetic women compared to non-diabetic women (Carati et al. 2013, 2668–74).

Among sexually active women, insulin-treated diabetic women were also more than twice as likely to report difficulty with lubrication and 80% more likely to report difficulty-achieving orgasm compared to non-diabetic women, after adjusting for the same demographic and clinical factors. When asked if their physical health limited their sexual activity, insulin-treated diabetic women were more likely than non-diabetic women to report that their health limited their sexual activity "quite a bit" or "extremely," in multivariable analysis (OR[95%CI] = 2.29[1.49–3.51]). However, non-insulin-treated diabetic women were not substantially more likely than non-diabetic women to feel that their health limited their sexual activity (OR [95% CI] = 1.29 [0.92–1.78]).

Diabetic women with peripheral neuropathy were also more likely to report less than monthly sexual activity, lower sexual desire, and limitation of sexual activity by physical health, compared to those without neuropathy. Among sexually active diabetic women, no significant associations between specific diabetic end-organ complications and sexual problems such as difficulty with arousal, lubrication, orgasm, or pain with intercourse were observed in adjusted models. There were also no significant associations between number of years since diabetes diagnosis and sexual function, after adjustment for end-organ complications (P > 0.05 for all).

In this cohort of ethnically diverse middle-aged and older women, diabetic and non-diabetic women reported similar levels of sexual desire and frequency of sexual activity, after adjustment for other demographic and clinical factors. However, both insulin-treated and non-insulin-treated diabetic women were significantly more likely to report low overall sexual satisfaction compared to non-diabetic women, and problems with lubrication and orgasm were more common among insulin-treated diabetic women compared to nondiabetic women. These findings suggest that while many diabetic women are interested and engaged in sexual activity, diabetes is associated with a markedly decreased sexual quality of life in women, either through complications of the disease itself or through utilization of treatments.

This study underlines the importance of distinguishing between different aspects of female sexual function when evaluating the burden of this disease. Based on this research, diabetes and its complications appear to have a much greater impact on sexual problems such as lubrication and orgasm as opposed to sexual desire or subjective arousal. Furthermore, our study indicates that the adverse effects of diabetes on sexual function may be concentrated in women taking insulin, an apparently high-risk group for developing sexual problems.

Diabetic women who were less motivated or interested in checking and controlling their blood sugars may have placed more priority on sexual activity and/or function in their daily lives, resulting in higher reports of sexual satisfaction. Alternatively, diabetic women with worse glycemic control may have had lower expectations about sexual activity in the setting of their poorly controlled disease, with the paradoxical result that they retained a stronger subjective sense of sexual satisfaction in spite of experiencing the same sexual difficulties. Differences in impulse control and other unmeasured personality factors could also have influenced both glycemic control and sexual satisfaction in diabetic participants.

Based on this research, clinicians may want to consider actively assessing for sexual problems in diabetic women, particularly those taking insulin, and counsel diabetic women that prevention of end-organ complications may be important in preserving their sexual function (Copeland et al. 2012, 331–40).

In Jordanian's study women were grouped into a diabetic married group (n = 613) and a nondiabetic married group (n = 524). The age of diabetic women ranged from 23 to 68 years (mean ± SD 46 ± 11 years), and the age of nondiabetic women ranged from 22 to 70 (51 ± 10 years). The prevalence of FSD in diabetic women 50 years of age or older was significantly higher compared with that in nondiabetic women. Desire, arousal, lubrication, and orgasm were more significantly affected in older diabetic women, whereas in younger women a significant difference was only found in desire. Multivariate analysis showed that glycemic control, type of diabetes, smoking, hypertension, dyslipidemia, and peripheral and autonomic neuropathy did not have a significant effect on FSD. On the other hand, longer duration of diabetes, older age, higher BMI, and the presence of coronary artery disease, nephropathy, and retinopathy had significant detrimental effects on female sexual function. This study shows that diabetic women in Jordan have more FSD than nondiabetic women. The prevalence of FSD in our study is in agreement with the global prevalence (Erol et al. 2002, 55–62) Neurovascular processes that mediate genital vasocongestion are impaired in diabetes (Meston and Frohlich 2001, 603–9) In this study, vaginal dryness was found significantly more often-in diabetic women than in nondiabetic women. Orgasmic dysfunction was more prevalent in older diabetic women in comparison with their nondiabetic counterparts. Kolodny reported a higher frequency of orgasmic dysfunction in diabetic women than in hospitalized women for various reasons (Enzlin et al. 2002, 672–77).

However, diabetic women were less satisfied with their sexual life. This is in agreement with the findings of Enzlin et al (Abu Ali et al. 2008, 1580–81).

Neuropathy, vascular impairment, and psychological complaints have been implicated in the pathogenesis of decreased libido, low arousability, decreased vaginal lubrication, orgasmic dysfunction, and dyspareunia among diabetic women. A cross-sectional study was conducted on 500 women who were recruited from a diabetes center, based on questionnaires completed by them. Data regarding demographic features, physical complications, and sexual disorders were obtained. Medical records of patients were used to obtain body mass index (BMI) and details of complications. Mean age of participants, duration of diabetes, and BMI was 48.8 ± 0.4, 8.9 ± 0.32 years, and 28.9 ± 0.23, respectively. Prevalence of sexual dysfunction was 32.3%. Low sexual desire was seen in 81.8%, disorders of arousal in 78.3%, of orgasm in 47.5%, and 35.1% had disorder in resolution area. There was no significant relationship between some factors such as age, duration of diabetes, BMI, and frequency of sexual dysfunction. Frequency of diabetic complications demonstrated a significant effect on the prevalence of sexual dysfunction. Sexual problems are frequent among diabetic women and deserve more attention in clinical practice and researches.

In a research conducted by Amini et al (Omidvar et al. 2013, 321–24) in Isfahan in 2001, a low sexual desire, lack of sexual satisfaction, low vaginal lubrication, and orgasmic dysfunction have been recognized as sexual problems among women. For every patient with diabetes who refers with a reduction in sexual desire, first other reasons (hormonal reasons and so on) should be ruled out, and then neuropathic; therefore, the genital organ should be examined, and the levels of testosterone, prolactin, thyrotropin, and estrogen should be

checked. Sexual dysfunction among women with diabetes includes vaginal dryness, a low sense of perinea, a lack of orgasm, and so on. The average age of the subjects, duration of married life, number of children, duration of diabetes, and body mass index (BMI) were 48.8 ± 0.04 years, 29.6 ± 0.5 years, 4.2 ± 0.1, 8.9 ± 0.3 years, and 28.9 ± 0.2, respectively. The results show a significant frequency of sexual dysfunction in different sexual areas. Majority (95%) of the subjects were suffering from type 2 diabetes; 26.3% of them mentioned a severe pain during sexual intercourse. In this study, there was no association between age, diabetes duration, and BMI with frequency of sexual dysfunction, although there was a significant association between age and dysfunction in desire (P = 0.02), sexual satisfaction (P = 0.01), and pain during intercourse (P = 0.004). It is found a significant statistical association between the number of complications due to diabetes and frequency of sexual dysfunction (P = 0.01). Overall, sexual dysfunction among women with diabetes was 32.3%. Nearly 82% of them were afflicted in the area of dysfunction in sexual desire, 78.3% had problems of arousal, 47.5% experienced dysfunction in orgasm, and 35.1% in the area of resolution. In all, 45.5% were not satisfied with sexual functioning. 39.4% experienced pain during intercourse, and 36.1% had disorders in vaginal lubrication. Previous reports have shown an increase in the prevalence of sexual dysfunction among women with type 1 diabetes (Omidvar et al. 2013, 321–24).

Conclusion

Diabetic women with end-organ complications such as peripheral neuropathy, renal dysfunction, stroke and heart disease were more likely to report decreased sexual activity or lower sexual satisfaction than diabetic women without these complications.

Several studies suggest the presence of sexual dysfunction among those who suffer from type two diabetes (Omidvar et al. 2013, 321–24; Bultrini et al. 2004, 337–40). These disorders include disorders of desire, lubrication, satisfaction, orgasm, and dyspareunia. The etiology of sexual dysfunction in patients with diabetes needs more attention because patients with diabetes are at risk for vascular and psychological complications. Therefore, they have a higher risk for developing sexual dysfunction (Fatemi and Taghavi 2009, 38–39).

These findings suggest that diabetic end-organ complications may play an important role in decreasing women's sexual quality of life, and that raise the possibility that prevention of diabetic complications may be helpful in preventing sexual dysfunction in women with diabetes.

The steps to be taken are clear: campaigns aimed at
(1) Prevention of type 2 diabetes
(2) Screening for early diabetic disease
(3) Increasing patient awareness of vulvovaginal disease
(4) Using medications of proven strategy and finally
(5) Researching and trialing of new therapies.

The ultimate challenge is to get action from primary health care to all higher levels; from the individual patient, to those at risk, in various health jurisdictions, in all countries despite varying economic circumstances and priorities. The problem is a global one and yet requires action at a local level; prevention, screening, and treatment strategies; education, including increasing awareness both in diabetic patients and those at risk of developing diabetes; and health priorities of governments. Basic research and clinical trials searching for a new understanding and therapies must be supported. It is time for strategies that prevent diabetes and its sequelae. It is time for programs for health care workers to diagnose and treat people with diabetic vulvovaginal disease, starting from basic hygiene that should be practiced with specific product, with specific active addressed to improve moisturisation, lubrication of the intimate area and able to exert a soothing action in case of irritability and itching.

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Developmental phonological dyslexia and dysgraphia in a regular orthography: a case study

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Abstract

In the Italian language the phoneme-to-grapheme mapping is fairly regular so that children are able to master very early reading and spelling skills. However, persisting phonological spelling deficits, were recently described in a sub-group of Italian dyslexic children with language delay.
The study describes spelling and reading deficits of an Italian child suffering from phonological dyslexia and dysgraphia. As a comparison, a case of surface dyslexia and dysgraphia was reported. Quantitative and error analysis were performed on reading and spelling performance. Additionally the locus of the phonological deficit was examined by means of tasks requiring doubled consonants processing. Results showed a parallelism between the reading and the spelling impairments in both children. Phonological dyslexia and dysgraphia was characterized by worse pseudoword than word processing; lexicalizations in reading and a high rate of minimal distance misspellings. Concomitant deficits in phoneme manipulation and representation were disclosed. The surface profile was characterized by impaired performance on tasks with pseudo-homophones and stress errors in reading, and concomitant phonological plausible misspellings.

Keywords: phonological dyslexia, phonological dysgraphia, consistent orthography, doubled consonants

Introduction

Developmental reading and spelling disorders are among the most common learning impairment (4-5% of the population, Consensus Conference ISS, 2011), with severe school consequences (Snowling, 1991). Over the past two decades, the understanding of these disturbances has grown thanks to cognitive models describing the normal processing of written language.
The two route model, originally formulated to describe reading (Coltheart, Patterson & Marshall, 1980; Patterson, Marshall & Coltheart, 1985) and later adapted for spelling (Beauvois & Dérouesné, 1981; Baxter & Warrington, 1985; Harris & Coltheart, 1986; Patterson, 1986), assume the existence of at least two procedure: a lexical procedure, along which words are processed as a whole (direct access) and a sub-lexical one, based on orthographic-to-phonological conversion rules in reading (and phonological-to-orthographic conversion rules in spelling) that exploits the systematic corre-spondences between phoneme-to-grapheme and vice versa. The sub-lexical spelling procedure is furthermore characterized by an acoustic-to-phonological conversion process that preventively segments and identifies the phonological string to be converted (Patterson, 1986).
Accordingly to this approach, studies on developmental dyslexia and dysgraphia described two main forms: a phonological and a surface disorder. Some children are selectively impaired in using the grapheme-to-phoneme conversion rules. Accordingly, they are impaired in reading novel words and pseudowords. This disturbance is generally called phonological dyslexia (e.g.,Temple & Marshall,1983). Other children make appropriate use of orthographic-to-phonological conversion rules and show a selective deficit in lexical access, with impaired reading of irregular words (e.g., yacht). Such a deficit is generally called surface dyslexia (e.g. Castles & Coltheart, 1993).
Similarly, developmental spelling deficits have been fractionated into phonological

(e.g.,Campbell & Butterwoth, 1985; Snowling, Stackhouse & Rack, 1986, Temple, 1986) and surface dysgraphia (e.g.,Temple, 1985; 1986). In phonological dysgraphia, correct spelling is possible for over-learned words, but not for less frequent or novel words and for pseudowords. In surface dysgraphia, there is an impaired spelling of irregular words and homophones (e.g. [diar] can be transcribed as either "dear" or "deer"). In both cases the correct transcription cannot be immediately deduced by applying the conversion rules. Errors are, for the most, phonologically plausible.

The distinction between phonological and surface disorders was developed on data from English and French, two language with quite irregular orthography. Generalization of this model (and of the predicted disorders) to language with more shallow orthographies such as Italian requires same considerations and a closer look at Italian orthography.

In Italian in the oral-to-written direction, the almost unique unpredictable condition is the site of word stress in three-syllable (or longer) words. However, stimuli allowing to test the lexical processing may be generated through pseudo-homophone contrasts. Italian dyslexic children are impaired in tasks with these stimuli, such as comprehension of sentence with pseudo-homophone contrasts (i.e. "l'ago" (the needle) or "lago"(lake), suggesting non-proficient use of the lexical procedure (Job, Sartori, Masterton, & Coltheart, 1983; Zoccolotti, De Luca, Di Pace, Judica, Orlandi, & Spinelli, 1999). Moreover, Italian dyslexic children have a selective deficit in judging the orthographic correctness of phonologically plausible pseudowords (e.g., the stimulus " *quore"[1], that is homophonic to the correct word "cuore", [kwore] (heart)). This difficulty is indicative of a prevalent reliance on a phonological procedure and a scarce use of the lexical one in performing the task (Marinelli, Angelelli, Notarnicola & Luzzatti, 2009; Angelelli, Marinelli & Zoccolotti, 2010). Moreover, different approaches such as eye movement recording and vocal reaction times have also been successfully used to study the reading processes in dyslexic children. Eye movement recording showed highly fractionat-

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1 The asterisk marks spelling errors that lead to pseudowords

ed text analysis, with a robust effect of word length (De Luca, Di Pace, Judica, Spinelli & Zoccolotti, 1999; De Luca, Borrelli, Judica, Spinelli & Zoccolotti, 2002). Consistent results were obtained from vocal RTs to different length single words (Zoccolotti et al., 1999; Spinelli, De Luca, Di Filippo, Mancini, Martelli & Zoccolotti, 2005; Zoccolotti, De Luca, Di Pace, Gasperini, Judica & Spinelli, 2005). Overall, psycholinguistic analysis of errors, eye movement recording and vocal RTs, indicated prevalent reliance on sub-lexical procedure. Thus, the efficiency of the lexical processing seems to be poor in Italian dyslexics, and they prevalently suffer from surface dyslexia (Zoccolotti et al., 1999; Marinelli et al., 2009).

Regarding spelling, the grapheme-to-phoneme correspondence is fairly regular in Italian; however, as in most other regular orthographies (e.g., Serbo-Croatian, Czech), there is a certain degree of ambiguity in the oral-to-written direction. For instance, the phonemic group [kw] may be transcribed by the orthographic sequences QU, CU, (for example, the Italian term for "rate" [kwota], is written "quota" and not "*cuota", whereas [kwoio], (lether) is spelt "cuoio" and not "*quoio"); no definite rule allows choosing among these alternatives and reference to a lexical entry is required (for a detailed description see also Zoccolotti, Angelelli, Judica & Luzzatti, 2005).

Using a spelling task that capitalizes on these oral-to-written ambiguities, a series of studies (Angelelli, Judica, Spinelli, Zoccolotti & Luzzatti, 2004; Angelelli, Notarnicola, Judica, Zoccolotti, & Luzzatti, 2010; Angelelli, et al. , 2010) reported a majority of surface dysgraphia in Italian dyslexic children, i.e. a selective impairment in writing unpredictable transcription words and many phonologically plausible errors.

What about the phonological forms of dyslexia and dysgraphia? The high correspondence between grapheme-to-phoneme in reading (and vice-versa in spelling) would suggest that the acquisition of such knowledge must be easy to learn in regular orthographies; indeed, in language such as Italian, cases with a deficient acquisition of the sub-lexical strategy are quite rare.

In fact, cross-linguistic studies indicate that literacy acquisition is not the same across lan-

guages and the facility depends on the degree of orthographic consistency of different languages. As to reading, Seymour, Aro and Erskine (2003), comparing the reading acquisition in different European languages, found that Italian children, at the end of their first year in primary school, were able to read in a proper way approximately 95% of a list of familiar words. Also regarding spelling (for reviews, see Caravolas, 2004) data support the view that, the sublexical procedure is acquired more rapidly in regular than in opaque orthographies. As regards Italian language two recent study comparing spelling (Marinelli, Romani, Burani & Zoccolotti, 2015) and reading (Marinelli, Romani, Burani, McGowan, & Zoccolotti, in revision) acquisition in Italian and English primary school children found greater accuracy in spelling and reading among Italian children than English children: former were very accurate after only 2 years of schooling, while among English children the performance was still poor after 5 years of schooling. Orthographic consistency produced not only quantitative, but also qualitative differences, with larger frequency and regularity effects in English than in Italian children, index of larger reliance on larger grain size in English sample. Even Orsolini, Fanari, Serra, Cioce, Rotondi, Dassisti, and Maronato (2003) found that by the fourth month of school 50% of Italian children were able to read approximately 80% of words correctly. For what regards spelling a recent study investigating spelling skills in first- to eighth-grade Italian normal readers (Notarnicola, Angelelli, Judica, & Zoccolotti, 2012) found that spelling along the sub-lexical conversion procedure was acquired quite early and ceiling effect was present for regular stimuli jet by third grade. Similarly, a study examining reading acquisition in Italian children from 2nd to 8th grade (Tressoldi, 1996) found very low percentage of errors already in the 2nd grade and smaller of 5% by 3rd grade. Overall, in consistent orthographies, conversion rules are easily acquired by almost children.

However, also in a very consistent orthography such as Italian, children may fail in the acquisition of phoneme-to-grapheme mapping. Literature reports a case of phonological dyslexia: Marco, a 12 –year-old Italian boy with a very evident deficit in reading pseudowords compared to words (Sartori & Job, 1983). With regard to developmental dysgraphia, a recent study (Angelelli, Putzolu, Iaia, Marinelli, Gasperini, Chilosi & Brizzolara, 2016) on Italian dyslexic children described long-lasting phonological spelling difficulties in those with a history of Language delay (LD). In particular, LD children were more sensitive to acoustic-to-phonological variables, showing relevant failure especially in spelling stimuli containing geminate consonants but also polysyllabic stimuli and those containing non-continuant consonants. Error analysis confirmed these results, with LD children producing a higher rate of phonological errors respect to NoLD children and controls. It could be worth to further investigate the uncommon cases suffering from phonological dysgraphia, taking into account potentially relevant factors such as concomitant deficits of phoneme discrimination, processing and representation.

The goal of the present study is to analyze the phenomenology of reading and spelling deficits of an Italian child suffering from a major deficit for phonological processing. As a comparison, the spelling and reading performances of child suffering from the specular disorder (i.e., surface dyslexia and dysgraphia) were also analyzed. Additionally, due to the specific difficulty of phonological dyslexic and dysgraphic children in dealing with geminate consonants, the processing of doubled consonants has been examined more deeply, with the aim to identify if the locus of the deficit may be phoneme discrimination, phoneme representation and manipulation and/or phoneme-to-grapheme conversion (and vice versa).

Method
Participants
The participant was A.S., a 10- year- old boy attending the fifth grade of southern Italy primary school. The child had great difficulty in reading and spelling when tested with a standard examination (see paragraph 2.3) although his intelligence, assessed with the Coloured Progressive Matrices of Raven (1984), was perfectly adequate for his age (score of 24, above the 10° percentile for this age level according to Pruneti et al., 1996).

A.S was indicated by his teachers as having significant difficulty in reading and spelling but he

received neither support for that problem. In fact, although his parents had been informed of A.S's poor performance, they did not take into account any rehabilitation program as they underestimated the seriousness of the problem and attributed to excessive teachers' worrying.

A.S. was very willing and collaborative in performing the various tests. On the discourse level no language problems were detectable, however parents referred a delayed development of language.

A.S's reading and spelling performance was compared to that of one child of the same age, G.S. The two boys were schoolmates and shared the reputation of problematic children. G.S., in fact, had marked reading and spelling deficits associated with normal socio-educational conditions and normal intelligence (score of 24 at the Coloured Progressive Matrices of Raven, above the 10° percentile for this age level according to Pruneti et al., 1996). G.S. was very collaborative in performing the various tests.

Reading assessment

Reading level was evaluated with a standard achievement test: the MT Reading Test (Cornoldi & Colpo, 1998). In this test, children were asked to read two passages. Speed (time in seconds per syllable) and accuracy (number of errors relative to the amount of text read) were measured from the reading of the first passage, which was read aloud with a time limit of 4 minutes. Comprehension was evaluated with the second passage, which could be read aloud or silently as preferred and without a time limit; after reading the passage, the child was required to answer ten multiple choice questions.

The nature of the reading disturbance of the dyslexic participants was also examined by additional tasks. The Words and Non-word Reading test (Zoccolotti, De Luca, Di Filippo, Judica & Spinelli, 2005) was administered. The test consists in four lists of words (varying for frequency and length) and two of pseudo-words (varying for length). Thirty stimuli per list were given; number of errors and reading speed were scored. The participants were required to read the stimuli aloud as quickly and accurately as possible. Number of errors and time (sec per list) were recorded and converted to z scores according to standard reference data (Zoccolotti et al., 2005).

Additionally, four tests of the Developmental Dyslexia and Dysgraphia Battery (Sartori, Job & Tressoldi, 1995) were adopted. In the Graphemes subtest participants were asked to name 21 single letters. It is useful to evaluate the efficiency of the transition from the grapheme to its phoneme. In the Lexical Decisions subtest children were required to read 48 stimuli and indicate if they were words or pseudowords. Homophones comprehension was assessed by 24 questions which required a choice among four possible answers (e.g.: "the needle (l'ago) is made of… water, wood, earth, metal"), two of them were possible if the target was processed phonologically. In order to answer the question correctly, children must be able to distinguish between "l'ago" (the needle) and "lago" (lake). Scoring is calculated with the following formula (Sartori, 1984): (homophonic errors / homophonic errors + correct answers) x 100. If a child scores around 50, he/she is not capable of distinguishing homophones, giving prove to rely on phonological processing. In the Correction of Homophones subtest children are asked to read 20 words of which only 8 are correct. The incorrect stimuli have been produced by inserting an apostrophe (e.g. "l'ametta" instead of "lametta" (blade)) or by segmentations of words (e.g. "di vano" instead of "divano" (sofa)). Both tests with pseudo-homophones were used to assess efficiency of lexical processing. Reading speed and number of errors were recorded in tests 1 and 3, while only the number of errors were recorded in the other two tests.

Spelling Assessment

Spelling abilities were tested through a standard spelling test (Angelelli, Marinelli, Iaia, Notarnicola, Costabile, Judica, Zoccolotti, & Luzzatti, 2016), composed of four sections:

Section A: regular words with complete one-sound-to-one-letter correspondence (N=70). Words were selected with different sources of phonetic-phonological complexity: (i) words made up of continuant sounds only (fricative, liquid or nasal consonants) versus words also containing non-continuant (plosive) consonants; (ii) words made up only of consonant-vowel (CV) syllables versus words also containing consonant clusters and doubled consonants;

(iii) disyllabic versus polysyllabic words. Different sources of phonetic-phonological complexity were used in order to determine variables influencing both segmentation and identification of phonemic string to be converted (for instance, continuant phones are, by nature, easiest to segment, and hence to identify, than non-continuant phones).

Section B: regular words requiring application of context-sensitive sound-to-spelling rules (N=10). In Italian, context-sensitive rules are required when spelling of a consonant depends on the following sound. For instance, the phoneme [k], is spelled C when followed by a consonant (e.g., CLIMA ([klima], climate) or by A, O, U (e.g., CASA [kaza], home) and CH when followed by E or I (e.g., BARCHE [barke], boats).

Section C: words with unpredictable transcriptions along phonological-to-orthographic conversion routine (N=55). This section includes: (i) words containing the phonemic group [kw], which in Italian may be transcribed by orthographic sequences QU, CU, or CQU; (ii) words containing syllables [t☐e],☐☐☐e☐, ☐dʒe☐, which may or may not require an I (e.g., [☐entsa], science, is spelt SCIENZA and not *SCENZA, while [☐ena], scene, is spelt SCENA and not *SCIENA); (iii) words containing plosive phones followed by liquid consonants [r] which are homophones to their doubled pairs (e.g., FEBBRE, fever and not *FEBRE, but LIBRO, book, and not *LIBBRO); (iv) words containing segments [lj] - [ʎ☐ and [nj] - [ɲ], that are homophonous in most Italian variants to the extent that [biljardo/biʎardo], billiards, is spelt BILIARDO and not *BIGLIARDO, while [folja/foʎa], leaf, is spelt FOGLIA and not *FOLIA; similarly [opinjone/opiɲone], opinion, is spelt OPINIONE and not *OPIGNONE, while [oɲuno/onjuno], everyone, is spelt OGNUNO and not *ONIUNO.

Section D: pseudowords with one-sound-to-one-letter correspondence (N=25). Items were controlled for different sources of phonetic-phonological complexity, as were words in Section A: (i) continuance of sounds (pseudowords with continuant versus non-continuant consonants); syllabic structure (pseudowords with consonant-vowel (CV) syllables versus pseudowords also containing doubled conso-

nants; and length (disyllabic versus 3-4 syllable pseudowords). Similarly to Section A, phonetic/phonological variables are introduced in order to account for variables influencing acoustic-to-phonological analysis that is preliminary to an effective phonological-to-orthographic conversion procedure.

Words and pseudowords were given in separate sequences and in a single quasi-randomized order. The examiner read each item aloud in a neutral tone, i.e., without emphasizing presence of clusters, doubled consonants or possible orthographic ambiguities. Children were asked to repeat each item before writing it down (so that the examiner could ensure that they had understood the item). When children failed to repeat or upon their request, the examiner read stimulus again. They were permitted to write in either capital or lower case letters. No feedback was provided on accuracy of written response. Final responses were counted, irrespective of correctness of first attempt. Children were tested individually. The number of correct spellings on each of the four sections of task was counted for every participant.

The DDO test allow also to perform an error analysis, in order to identify nature of spelling errors, irrespective of section of test in which they emerged. Errors were coded as:

-Phonologically plausible errors (impaired spellings along lexical route): spelling errors that can be pronounced to sound like target words; these errors arise from over reliance on phoneme-to-grapheme conversion routine (e.g., "*cuota" instead of "quota" (rate));

-Phonologically non-plausible errors (inaccurate spellings via sublexical routine): errors causing a change in phonemic makeup of a word reflecting difficulties in phonemic segmentation, phoneme-to-grapheme encoding or a phonological/graphemic buffer disorder. This category included the following error subtypes:

- Errors based on minimal distance features: substitutions of consonants or vowels with other consonants or vowels that differs only in one single distinctive feature [e.g., sonority, "fino"(until) instead of "vino"(wine); continuance, "pesta"(crush) instead of "festa"(holiday)]. Doubling of a single consonant or of dedoubling of a doubled consonant were also considered in this category;

- Other errors: non-minimal-distance substitutions (e.g., "*balo" instead of "baco" (worm)), omissions (e.g., "*vsone" instead of "visone"(mink)), insertions (e.g., "*manrmo" instead of "marmot"(marble)) and letter transpositions (e.g., "*patro" instead of "prato"(field)).
- Context-sensitive sound-to-spelling errors: errors in application of context-sensitive sound-to-spelling rules (e.g. "*adago" instead of "adago" (slow) or "sceda" instead of "scheda"(card)).

For both quantitative and qualitative data, the performance of each participant was compared to reference data (Angelelli et al., 2016).

Phonological Awareness Assessment
Phonological awareness was evaluated with a blending test (Di Filippo, Brizzolara, Chilosi, De Luca, Judica, Pecini, Spinelli, & Zoccolotti, 2005). The test consisted in asking the child to repeat aloud the whole word (or pseudowords) which results from the blend of "sounds" emitted at a frequency of one per second, for a total of 19 words and 19 pseudowords (composed of 5-6 letters). For each item, both the number of correct blended pairs of phonemes and the number of the whole correct blended items were recorded. Before the test itself, a number of warm-up tests were run to help the child understanding the nature of the main test.

Processing of doubled consonants
Additionally, due to the A.S. difficulty's in dealing with doubled consonants, the child was tested with an experimental protocol prepared ad hoc. The aim was investigating if phonological difficulties in processing stimuli with doubled consonants were due to inefficiencies of: i) phoneme discrimination, ii) phoneme manipulation, iii) phoneme-to-grapheme conversion (and vice-versa).
We selected 20 pairs of real words differing for the presence of doubled vs single consonants (e.g., "pala-palla" (shovel-ball)). We also created 20 pairs of pseudowords (one with a doubled consonant, and one without, e.g., "pasu-passu"), for a total of 80 stimuli. In both the word and the pseudoword pairs, half of the doubled consonant were continuant phones (i.e., the fricatives [f], [v], [s], [S], the liquid [l], [r] and the nasal consonants [n], [m], [ñ], [N]) that are susceptible to prolongation) and half

non-continuant consonants (plosive consonants, such as [p], [b], [t], [d]). In fact, acoustic-to-phonological analysis is subject to acoustic-to-phonological quality of phones in dyslexic children (especially for those with a phonological deficit, Angelelli et al., 2016). It is easier to isolate, segment and identify continuant phones than non-continuant phones (see also Luzzatti, Colombo, Frustaci & Vitolo, 2000 for coherent data on aphasic patients).
All the selected stimuli were presented in four tasks. In particular, in order to examine the acoustic-to-phonological discrimination, as well as the phonological buffer and retrieval of the phonological output, a repetition task was used. In this case, A.S. listened each stimulus and after repeated it. The ability of manipulate phones was examined through phoneme segmentation and blending tasks. In the blending task, words or pseudowords were presented phoneme-by-phoneme at a rate of one per second. At the end of the sequence, the child was asked to repeat aloud the whole stimuli. On the contrary, in the segmentation task, the examiner pronounced the whole stimulus and the child was asked to segment into the single phonemes constituting the stimulus. The phoneme-to-grapheme conversion skills were tested through an acoustic-to-visual matching task: the child listened a stimulus and was asked to choose the correspondent transcription between a pair of stimuli, one containing the doubled consonants, the other one did not. The stimuli appeared in a sheet in front of him. To avoid attentional lapses, each task was performed in a different day.
Only attended errors regarding doubled consonants processing were computed; while other errors were not analysed.[2] Self-correction were accepted.

Procedure
Children were tested individually in a quiet room. Parents were informed on the screening activity and authorized the participation to the study by signing the appropriate informed consent paperwork.

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[2]Other errors were 12.5% in the repetition, 7.5% in segmentation and 2.5% in blending task; in the visual-acoustical matching it was not possible to make other errors except those on doubled consonants

Data analysis

Regarding reading performance, the number of errors and the reading speed were analysed respect to proper normative data and transformed into z scores. The performance on Single words and non- word -reading test (Zoccolotti et al., 2005) was futhermore analysed by applying a Multivariate Logistic Regression Analysis (Aitkin, Anderson, Francis & Hinde, 1989) in order to compare accuracy reached in words vs pseudowords. The dependent variable was the accuracy to each item (1 = passed, 0 = failed); independent variables were lexicality (1 = words; 0 = pseudowords), word length (number of letters), presence of clusters (1 = item with cluster; 0 = item without cluster) and presence of doubled consonants (1 = item with doubled consonants; 0 = item without doubled consonants).

An error analysis was also performed on children reading performance at Words and Non-word Reading test (Zoccolotti et al., 2005). In particular errors were coded in: 1) stress errors; 2) visual/phonological errors (more than 50% of letter in common with the target stimuli) producing a pseudoword; 3) visual/phonological errors (more than 50% of letter in common with the target stimuli) producing an existing word; 4) words semantically related to the target words.

With regard to the spelling task, the number of items transcribed correctly in the four sections of the test were computed and transformed in z score according to normative data (Angelelli et al., 2016). In addition, the spelling performance was analysed by Multivariate Logistic Regression Analysis (Aitkin et al., 1989). Based on psycholinguistic models that assume two independent spelling procedures, the ability of each participant in spelling regular words was compared with that in spelling words with an unpredictable orthography (as a marker for the lexical spelling route) and pseudowords (as a marker for the sub-lexical spelling routine). Two logistic regression analyses were applied to the profile of each participant: the first compared regular words versus words with unpredictable transcription; the second, regular words versus pseudowords. The units were the items of the spelling test (125 for the first comparison, 95 for the second one); the dependent variable was dichotomous (pass or fail).

Potentially relevant variables such as frequency, length (number of letters), cluster presence (1=item with cluster; 0=item without cluster) and the presence of doubled consonants (1=item with doubled consonants; 0=item without doubled consonants) were checked in both analyses. As reported in the spelling session, errors were also classified according to Angelelli et al. (2016). For each participant the number of errors for each typology was compared to normative data.[3]

Regarding the experimental paradigm on doubled consonants processing, accuracy in the different tasks was compared by a Multivariate Logistic Regression Analysis (Aitkin et al., 1989). The dependent variable was the accuracy of A.S. in processing each item (1 passed, 0 fail), and the independent variable was the task (repetition vs segmentation, blending and acoustic-to-visual matching respectively; segmentation vs blending and acoustic-to-visual matching; blending vs acoustic-to-visual matching). Additionally, in order to assess the effects of lexicality and acoustic-to-phonological variables on accuracy, lexicality (1 = words; 0 = pseudowords), presence of continuant sounds (1 = item with continuant consonants; 0 = item with non continuant consonants) and presence of doubled consonants (1 = item with doubled consonants; 0 = item without doubled consonants) entered in the analysis as independent variables. Moreover, analysis were replicated separately for words and pseudowords in order to check for different pattern in lexical vs non lexical stimuli.

Results

Reading

The performances of the two children on the MT Reading Tests (Cornoldi & Colpo, 1998) are reported in Table 1.

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[3] Note that, in the error analysis, for both subject we classify quality of error irrespective of stimulus sub-test. It is obvious that that the probability to commit phonological plausible errors is lower compared to conversion errors. In fact phonological plausible errors are possible mainly for irregular words; on the contrary conversion errors are possible for each grapheme constituting words and pseudowords.

Table. 1. Performance of G.C. and A.S. on the MT reading Test (Cornoldi & Colpo, 1998). Values in brackets refer to z score according to normative data. In all instances negative values represent a poorer performance.

	G.C.	*A.S.*	**Normative sample Mean d.s.**
sill/sec	2.15 (-1.3)	0.75 (-2.42)	3.77 1.25
number of errors	20 (-2.27)	27 (3.4)	5.9 6.2
Comprehension: Correct answers	4 (-1.64)	8 (0.18)	7.6 2.2

A.S. did not manage to finish reading the passage in the fixed time, making a high number of errors (27 errors, his performance was 3.40 standard deviations below the normative sample), which is almost 4 times the number made by normal readers of same age (according to normative data). He read with a speed of 0.75 sill./sec. against the 3.77 sill/sec expected for his age and grade (on average he was -2.42 standard deviations below the normative sample). In fact, he read very slowly, taking several pauses over 5 seconds due to difficulty in decoding complex graphemes. Overall, his performance placed him, both for speed and accuracy, in the category of children requiring "immediate help". On the comprehension task, after a long (30 minutes) and arduous process, Albert managed to extract from the text the information necessary to reply correctly to 8 out of 10 questions (z = 0.18).

G.C. made 20 errors when reading his passage (z = 2.27); on average he read 2.15 syllables in one second (z = -1.3). His comprehension skills were impaired (only 4 correct answers out of 10; z = -1.64). Comparison to normative data, his performance fell in the category of children requiring "immediate help for speed, accuracy and comprehension.

Figure 1 reported z scores obtained by G.C. and A.S. on the Developmental Dyslexia and Dysgraphia Battery (Sartori et al., 1995).
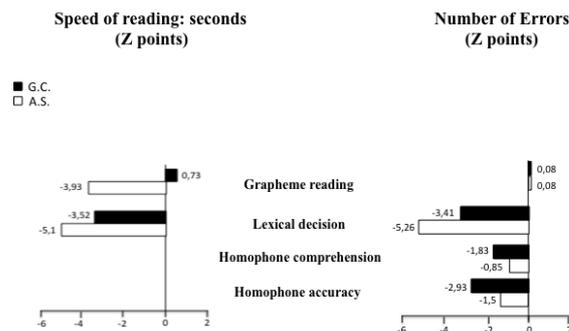

Figure. 1. Performance of G.C. and A.S. on the *Developmental Dyslexia and Dysgraphya Battery* (Sartori et al.,1995). In all instances negative values represent a poorer performance.

A.S.'s performance in grapheme reading was impaired for speed, taking 24 seconds without, however, making any errors. His performance was 3.93 standard deviations below that of controls.

He also underperformed on lexical decision task (approximately - 5 standard deviations below norms for both errors and time). Here A.S. made 17 misjudgments and took twice the time of controls (193 seconds).

However, in pseudo-homophone tasks, A.S performed as controls both in comprehension (z = -0.85, only 2 homophonic errors) and correction (z = 1.5, only 3 errors), showing discrete lexical access.

Single word and pseudoword reading was impaired for all stimuli categories and for both speed and accuracy (see table 2). However, it is interesting to note that A.S. scored 67% on words and 33% on pseudowords. The difference between the performance on words and pseudowords is 34%. When comparison is limited to long words and pseudowords, the difference becomes 38%, which is very close to the critical threshold proposed by Sartori (1985) as an indication of phonological dyslexia.

The logistic regression analysis (see table 2), confirmed a significant lexical effect ($\chi^2 = 8.13$, p < .01), with pseudoword spelled significantly worse than words. He showed a word length effect too (p < .01).

Table 2. Performance of G.C. and A.S. on the Words and Non-word Reading test (Zoccolotti et al., 2005). Values refer to z score according to normative data.

	Accuracy		Speed	
	A.S.	G.C.	A.S.	G.C.
Short high frequency words	7.87	2.5	20.31	1.01
Long high frequency words	5.41	3.83	12.07	1.52
Short low frequency words	6.63	6.02	17.49	0.72
Long low frequency words	7.75	4.06	13.31	1.41
Short pseudowords	7.18	2.82	14.66	0.81
Long pseudowords	14.6	3.68	10.92	0.27

On the contrary, G.C.' grapheme reading was normal for errors and time. He took 10 seconds (z= -0.73), without making any error.

He underperformed on lexical decision task, approximately 3.5 standard deviations below controls, for both errors (12 misjudgments) and time (he concluded the test in 159 seconds, almost twice the time taken controls).

G.C had also difficulties on pseudo-homophone comprehension (z= -1.83 SD) and correction (z= -2.93 SD). In the comprehension task, in fact, he chose the homophonic alternative in 61.9% of cases, showing a main reliance on phonological processing (he interpreted both "s'offre" (he offers) and "soffre" (he suffers) as "sta male" (he is sick); both "vera" (true) and "v'era" (there was) as "reale" (real)). The limited number of non-homophonic alternatives (n= 2) showed that the performance itself was valid and that there was no general comprehension problem (Sartori, 1984). G.C. made a total of 5 misjudgments on pseudo-homophone correction, for example accepting "di vano" for "divano" (sofa), "lerba" insead of "l'erba" (grass).

In single word and pseudoword reading, G.C. was below the mean for accuracy in all stimuli categories (see table 2). The logistic regression analysis showed not significant effect of lexicality ($\chi^2 = 1.70$, ns): words and psudowords were comparably impaired. The only variable that significantly affected his performance was stimulus length (p< .01).

From a qualitative point of view, we can observe that A.S. did not commit any stress errors. He made prevalently visual/phonological errors (sharing more than 50% of letters with the target stimuli) leading to pseudowords (31.6%) and word substitutions semantically/visually related to the target word (10.6%). It is clear that A.S. had difficulties in phonological decoding, that tried to compensate by an attempt to retrieve the whole word phonology. Morphological errors and errors producing a word unrelated to the target were both about 1.5%.

At the contrary, G.C. made many stress errors (4.2%; note that in the test there were few words irregularly stressed, then this percentage is relevant). The percentage of visual/phonological errors (sharing more than 50% of letters with the target stimuli) leading to pseudowords, and word substitution (semantically or visually related) were substantially lower than those reported by A.S. (20% and 3.9% respectively for the two errors). Also for G.C., morphological errors and errors producing existing words, unrelated to the target, were both about 1.5%.

Brief summary

The patterns of reading impairment that emerged from children's performance are complex but suggest that A.S. is affected by phonological dyslexia while G.C. suffers from surface dyslexia.

Different characteristic emerged for the two children about the nature of their reading deficits. A.S. performed adequately on pseudo-homophone tests but was impaired on the other tasks, including the reading of single graphemes. A lexicality effect emerged when reading lexical vs non lexical stimuli: pseudowords, in fact, were read significantly worse than words. From a qualitative point of view, he made no stress errors, indicating a certain command of lexical procedure, and numerous lexicalizations, suggested an incorrect attempt to use the lexical procedure. On the contrary, G.C. displayed the characteristic of surface dyslexia. In fact, the performance on the various tests are indicative of reliance on phonological rather than lexical procedure. He underperformed on pseudo-homophone tests (correction and comprehension), indicating lexical reading deficit, but single grapheme reading was normal. Moreover,

no lexical effect was present: word and pseudoword reading was comparably impaired. The analysis of reading errors confirmed a sub-lexical pattern, as he made many stress and visual-semantic errors.

Spelling

Compared to normative data, A.S. had greater difficulty in spelling pseudowords ($z = -10.17$) and regular words ($z = -8.81$ for words with one-sound-to-one-letter correspondence and $z = -2.43$ for words requiring syllabic conversion). The performance was not adequate neither in spelling words with unpredictable transcription ($z = -3.64$).

However, results at the logistic regression analyses showed that A.S. had a comparable level of accuracy on both regular and unpredictable transcription words ($X^2 = 5.34$, ns). However, he presented a significant lexical effect ($X^2 = 5.80$, $p < .05$): pseudowords were spelled worse than regular words. With regards to other variables taken into consideration, none appears to have a significant effect with the exception of presence of doubled consonants ($p < .0001$).

On the contrary, G.C.'s level of accuracy on spelling regular words and pseudowords was good ($z = -0.69$ and $z = -0.17$ respectively), but his performance on unpredictable transcription words and words with syllabic conversion was impaired ($z = -2.24$ and $z = -6.71$ respectively). Logistic regression analysis confirmed a significantly effect of regularity ($X^2 = 10.15$, $p < .01$): words with unpredictable transcription were spelled significantly worse than regular words, while accuracy on words and pseudowords was comparable ($X^2 = 1.80$, ns). No other variables affected spelling.
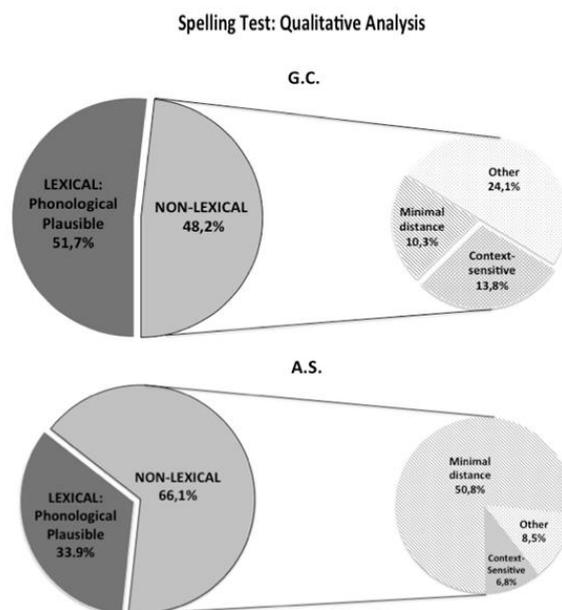

Figure 2. Typology of errors made by the two children on the spelling task.

As reported in Figure 2, A.S. committed prevalently phonological errors (66.1%). In particular, the most were minimal distance errors (50.8%; $z = 20.97$) and in particular doubling of a single consonant or dedoubling a doubled consonant. He also made many syllabic conversion errors (6.8%, $z = 4.74$) and simple conversion ones (8.5%, $z = 3.84$). Also phonologically plausible errors were not negligible (33.9%, $z = 3.39$).

On the contrary, most of the errors made by G.C. were phonologically plausible (51.7%, $z = 2.12$). However, minimal distance (10.3%, $z = 1.41$), syllabic conversion (13.8%, $z = 4.74$) and simple conversion (24.1%, $z = 5.71$) errors were also present.

Brief summary

Both the quantitative and the qualitative spelling analyses suggest a major impairment of the sub-lexical procedure in A.S. He presented greater difficulty with pseudowords compared to regular words and made numerous minimal distance misspellings, in particular on doubled consonants. However, in comparison to controls, it appears that the lexical procedure was not wholly efficient either.

On the contrary, G.C. suffered from an inefficient use of the lexical spelling procedure, while the sub-lexical one was better. In fact, he showed a selective impairment in spelling unpredictable transcription words, whose correct

spelling require access to lexical orthographic representations. Moreover, error analysis revealed a prevalence of phonologically plausible errors, confirming a good knowledge and command of phonological processes.

Phonological Awareness

Figure 3 reports children' performance on the blending test (Di Filippo et al., 2005).

A.S.'s phonological awareness was impaired. In the word condition, he only managed to correctly produce 11 out of 19 items (58%) and made 61 blends out of 83 (73.5%). His performance was 1.6 standard deviation below controls on both items. With pseudowords, A.S. was significantly worse: he produced only 1 pseudoword correctly out of 19 (5.3%) and 44 blends out of 83 (53%). His performance was 4 standard deviations below controls.

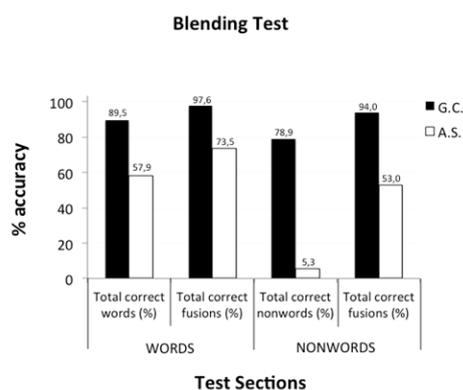On the contrary, G.C.'s performance was spared for each blending measure examined.

Figure 3. Percentage of accuracy on the Blending Test (Di Filippo et al., 2015)

Experimental tasks on doubled consonants processing

Accuracy of A.S. on the various tasks tapping doubled consonants processing was higher for repetition (93.7%) respect to phoneme segmentation (42.5%), phoneme blending (46.3) and visual-acoustic matching (65%). Logistic analysis confirmed significantly higher accuracy in the repetition than in each other task (at least p < .0001). Phoneme segmentation and blending did not differ (X^2 = 0.24, ns) but both were performed with lower accuracy compared to visual-acoustic matching (at least p < .01). In each analysis the presence of doubled consonants significantly affected performance (at

least p < .01), except for the analyses comparing repetition vs visual-acoustic matching and repetition vs phoneme blending. In fact during the repetition task, the child had similar performance with stimuli containing doubled vs single consonants (95% and 92% respectively). Similarly, in the visual-acoustic matching task, he reached 70% and 60% of accuracy with stimuli containing single vs doubled consonants. On the contrary, in meta-phonological tasks (phoneme segmentation and blending), accuracy depended from the presence of doubled consonants. In fact, in segmentation A.S. tended to produce always stimuli without geminate: he obtained 80% of accuracy on stimuli containing single consonants and only 5% on stimuli with doubled ones (that tended to segment always without geminate). In blending task, he tended to produce the stimuli with geminate: the accuracy was 63% and 30% on stimuli with doubled vs single consonants, respectively. A significant lexicality affect emerged in the comparison between repetition and visual-acoustic matching (p < .05).

In order to deeply examine the lexicality effect, separate analysis on words and pseudowords were carried out. Accuracy of A.S. on the various experimental tasks, separately for words and pseudowords, is reported in Figure 4.
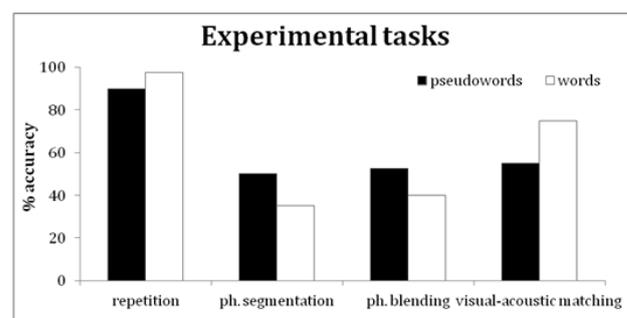
Figure 4. Performance of A.S. on experimental tasks tapping doubled consonants processing.

With words, all tasks' comparisons were significant (at least p < .05; for the direction of effect see the later paragraph), except for phoneme blending and segmentation that were performed with similar accuracy. The unique controlling variable significant was the presence of doubled consonants in phoneme segmentation vs repetition and visual-acoustic matching comparisons (X^2 = 9.48, p < .05; X^2 = 5.57, p < .01; respectively). With pseudowords, the performance in visual-acoustic matching wors-

ened and reached an accuracy similar to that highlighted in meta-phonological tasks (phoneme bending and segmentation). The presence of doubled consonants affected the performance in each comparison (at least p < .05), except for the analyses comparing visual-acoustic matching vs repetition and phoneme blending. Moreover, comparing visual-acoustic matching vs repetition and phoneme segmentation, also continuance of sounds was significant (at least p < .05).

Brief summary

A.S.' performance in tasks tapping doubled consonants processing highlighted that difficulties were not limited to phoneme-grapheme mapping (investigated through a visual-acoustic matching test), but interested also phoneme bending and phoneme segmentation abilities. Then, it seems that the ability to represent and manipulate phonemes was impaired. On the contrary, repetition was good showing normal sensory elaboration as well as normal phonological output retrieval. Performance in the visual-acoustic matching worsened with pseudowords, probably due to the impossibility to compensate phonological difficulty through lexical processing. Moreover, with non lexical stimuli, also the presence of non continuant consonants significantly affected A.S.' performance.

Discussion

The study reports the case of a child suffering from developmental phonological dyslexia and dysgraphia, as for control a second case displaying the characteristics of surface dyslexia and dysgraphia.
Most Italian dyslexic children have been described as suffering from surface dyslexia (Marinelli et al., 2009, Zoccolotti et al., 1999) and dysgraphia (Angelelli et al., 2004, Angelelli et al., 2010), since they showed a prevalent reliance on phonological procedure as highlighted by impaired lexical reading and defective irregular word spelling, with many phonological plausible errors. The reading and spelling performance of G.C. was coherent with a diagnosis of surface dyslexia and dysgraphia. G.C. showed a selective impairment in spelling words with unpredictable transcription, made mostly phonologically plausible errors with better transcrip-

tion of regular one-phoneme-to-one-grapheme stimuli. Similarly, in reading G.C. relied on phonological rather than lexical procedure: he underperformed in task tapping the lexical procedure such as correction and comprehension of pseudo-homophones. Moreover, his phonological awareness was adequate, as demonstrated by good meta-phonological abilities. On the contrary, A.S.'s deficits seemed prevalently due to significant inefficiencies of the sub-lexical procedure. This would indicate, on the one hand, that also in a relatively shallow language, the acquisition of phoneme-to-grapheme mapping may be difficult in some children, despite the its easiness in normal development (e.g., Orsolini et al., 2003; Tressoldi, 1996; Seymour et al., 2003; Marinelli et al., 2015, Marinelli et al., in revision). Given the significant difficulties that A.S. was still having in the fifth year of primary school, we consider possible that he might have adopted a lexical strategy to compensate his problems. This strategy, however, deprived by normal sub-lexical procedure development, remained incomplete. In fact, A.S. performed adequately on homophone tests, but underperformed on the other tasks being poor also in single graphemes reading. He showed a lexicality effect in reading, with worse performance in pseudoword compared to stimuli with a lexical status such as words. His reading errors were mainly phonological errors, i.e. inefficiencies of the grapheme-to-phoneme conversion (that in almost case generate pseudowords), while no lexical errors (i.e. stress errors), were detectable. In pseudoword reading A.S. made also numerous lexicalizations, as well as visual and semantic errors (words semantically and visually related to the target word). This could suggest incorrect attempts to overuse the lexical procedure in order to compensate phonological decoding difficulties. In spelling, both the quantitative and the qualitative analyses, suggested a main impairment along the phonological procedure: he had greater difficulty with pseudowords compared to regular words and made numerous minimal distance misspellings.
The phenomenology of A.S.' spelling deficit is coherent with what found in a recent study (Angelelli et al., 2016) examining developmental dysgraphia in Italian dyslexic children with a history of Language Delay (LD). These children produced a higher rate of phonological errors

respect to children without LD and controls and were more sensitive to acoustic-to-phonological variables, showing relevant failure especially in spelling stimuli containing geminate consonants but also polysyllabic stimuli and those containing non-continuant consonants. In this study we did not directly test A.S. linguistic skills, however we can suppose, retrospectively, a history of language delay.

To summarize, in Italian, the high consistency of orthographic mapping render the acquisition of the phoneme-to-grapheme and grapheme-to-phoneme conversion rules very easy. In fact almost all children master the phonological processing very early (Orsolini et al., 2003), and Italian dyslexic and dysgraphic children show prevalently a defective lexical procedure acquisition (Angelelli et al., 2010). However, also the acquisition of sub-lexical mapping may be difficult, especially in children with concomitant language weakness. For this reason it is very important for therapists and teachers to take into consideration this possibility and to promote a strengthening of reading and spelling skills in children with a history of language deficit. Moreover, an open question remains if cases of phonological reading and spelling deficits might occur also in absence of a history of language deficit.

Regarding the A.S.'s difficulty in processing stimuli with doubled consonant, results highlighted that phonetic-to-phonological variables such as the presence of doubled consonants and also continuance of sounds affected the phonological-to-orthographic (and vice versa) conversion (for coherent data see Angelelli et al., 2016). Also the lexical status of stimuli affected the performance, with better performance on words compared to pseudowords. This latter phenomenon might be due to greater difficulties in retaining stimuli without lexical status (absent in the phonological lexicon), as well as to the impossibility to compensate phonological decoding difficulties by a lexical support. Moreover, results highlighted also that phonological difficulty in processing stimuli with doubled consonants was not limited to phoneme-to-grapheme (and vice versa) conversion. A.S., in fact, underperformed not only in the visual-acoustic matching but also in tasks requiring phonological awareness such as phoneme segmentation and phoneme blending.

However, the spared performance in repetition seemed to exclude the possibility of acoustic discrimination deficits, as well as deficits in the phonological output retrieval. On the other hand, jet Marinelli, Di Filippo, Angelelli and Zoccolotti (2011) failed to find a deficit in repetition in Italian dyslexic children (while the performance in reading the same stimuli presented visually was severally impaired). Data were coherent with those on English dyslexic children (Ramus et al., 2003) reporting that a phonological deficit can appear in absence of sensory disorders.

The question regarding the functional locus of phonological reading and spelling deficits remain debated. Some authors support the hypothesis of a primary sensorial deficit which involves the elaboration of both linguistic and non linguistic stimuli (Tallal, Miller, Bedi, Byma, Wang, Nagarajan, Schreiner, Jenkins, & Merzenich, 1996); others support the hypothesis of a phonological coding/decoding impairment, relative to the extraction of phonological invariants from an acoustic flow (e.g., Liberman, 1998). Alternatively the locus of phonological spelling and reading difficulties may be linked to phonological awareness deficits. In the present study we have deeply examined only the ability to process stimuli with doubled consonants, and data do not support a sensory impairment (as highlight by the good performance of A.S. in the repetition task) but indicate both phonological awareness and phonological-to-orthographic conversion deficits.

Moreover, it is interesting to note that both children showed concomitant dyslexia and dysgraphia as well as a closer parallelism between reading and spelling deficits. Comorbidity and identical direction of the two deficits suggested the use of the same strategy for reading and spelling. Regarding lexical deficits, several studies (see Angelelli et al., 2010 for data on Italian children) have found, through an item-by-item analysis, that when children have the orthographic representation of a word in their lexicon, they use the lexical procedure regardless of the task (reading or spelling). Conversely, when this representation is unavailable, both reading and spelling are impaired. Data support the hypothesis of a unique orthographic lexicon, used for both reading and spelling in dyslexic and dysgraphic children as well as in young normal

reader (Allport & Funnell, 1981; Behrmann & Bub, 1992; Coltheart & Funnell, 1987). Regarding phonological deficits, it is clear that children that do not master the phonological processing have impaired performance in both oral-to-written and written-to-oral conversion, and that performance will be affected by the same variables in reading and spelling. These findings also have educational/clinical implications, because it appears that training reading ability can improve spelling ability, and vice versa. This can be advantageous for learning (Conrad, 2008; Ehri, 1980; Ehri & Wilce, 1986; Share, 2004) and rehabilitation. In fact, several developmental studies, have reported generalization of reading treatment effects to spelling and vice versa (Brunsdon, Hannan, Coltheart & Nickels, 2002; Lorusso, Facoetti, & Molteni, 2004; Lorusso, Facoetti, Paganoni, Pezzani, & Molteni, 2006; Brunsdon, Coltheart, & Nickels, 2005).

In conclusion the present study examined a rare case of phonological dyslexia and dysgraphia in Italian, a very consistent orthography, highlighting the role of concomitant deficits of phoneme representation and phoneme manipulation.

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Dementias in a systemic view a survey in Brindisi social health service district

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Introduction

The natural world is still considered a large complicated machine, of which we can have linear explanations, knowing its functioning mechanisms. Human organizations as well are considered a complicated gear: for this reason actions are taken in order to increase control systems and tools, to make the procedures more specialised, to produce more advanced software, to engineer the type of analysis. However, an organization – besides goods and services – produces meaning: a combination of meaning sometimes coherent, sometimes contradictory and chaotic imposes itself among social players, but that emerges from the interaction between them. Although it is the basis of every social form (Luhmann 1995), communication – and the related production of meaning – is taken for granted and therefore misjudged. The communicative analysis is crucial to the extent that it is an expression of shared working practices among subjects with different perspectives, languages, experiences and knowledge. In different fields of study – sociology and occupational psychology, managerial sciences – recent investigations show how human organizations set up a complex system, where beside the official organizational and regulatory structure (top down), a latent informal structure is strongly attested, which is formed in a context of constant relationships (bottom up), in which the subjects perceive the working environment, themselves, their role, norms, values and the other (Alvesson and Berg 1993; Argyris 1994; Piccardo 1995; Piccardo and Benozzo 1996).

Based on the perspective of complex systems, in 2012-2013 a group of sociologists of the Salento University carried out a survey on the issue of people with dementia and Alzheimer's and their relationship with health care facilities[1]. In order to prepare a program of interventions in some sectors of the Local Health Authority – with consequences on families and associations interested in the issue – and to provide training for health employees and patients, the general aim of the research project was to get information on three areas: the environment that surrounds people with dementia (health and associative resources in the area); the system of action of families in which there is a patient with dementia/Alzheimer's; the interaction between them and the health care facilities.

Before the explanation of the survey outcomes, the perspective of complex systems, especially in the social sphere, and the aspects considered will be clarified.

A less reductive vision in order to know the reality

Much of the order we observe is spontaneous, a natural expression of the surprising capacity of self-organization which is in the universe, considered as a complex network of relationships (Kauffman 2001). After the research of Ilya

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[1] The action-research "Voglio vivere" – decided by the Local Health Authority ASL in Brindisi – had a research team consisting of 9 sociologists and a statistician. The undersigned has assumed the role of scientific director and coordinator of the research group, building tools and choosing methods and techniques of the various phases of the survey. A psychiatrist and a trainee, graduated in psychology, supported the investigation on behalf of the Local Health Authority.

Prigogine, many scholars analyse the reality through the approach of complex adaptive systems (biological and social): characterised by numerous and different elements and multiple connections, they try to adapt by maximizing their evolutionary possibilities. In order to do it, systems self-organize: self-organization is an essential feature of complex systems and a prerequisite for the ability to evolve, since it generates adaptive parts and structure in a selective way. In the field of natural selection, self-organised features can be moulded more quickly because self-organisation emerges from below and has consequences on (and produces) higher hierarchical structures (bottom-up) (Colombo, 1991; Bocchi and Ceruti 1985; Capra 2001).

Self-organization depends on the close connection between elements, so that the result is much more than the sum of its parts; these parts must be considered within a set of relationships that determine significance and position of each component. As a result the systems may be, as the conditions change, in a dynamic intermediate situation between a completely predictable one and another which is chaotic and unpredictable. In this situation, the system shows "emerging" properties: between order and chaos there is the sudden appearance of unexpected regularities. While complicated systems (an assembly line or internal combustion engine) are made up of a set of elements, whose functioning is predictable and can be organised – it consists of a series of processes and straight chains of cause and effect (A→B→C→D...) – complex systems (weather, brain, families, stock markets, hives, companies) are characterized by non-linear dynamics. For those systems it is noticed the presence of an internal order that can not be inferred a priori, because, although stimulated from the outside, the systems create new dynamics in an endogenous way through self-regulation mechanisms. It means that each system, although in need of resources from the environment of reference, it is capable of producing by itself the elements which are necessary for survival (autopoiesis) through regularities and its own operating laws (self-referentiality): if a man eats a chicken, he does not become a chicken, but it is the chicken that is transformed into the elements necessary for the life of the human organism. Moreover, be-

sides the ability to adapt, the success of a system depends on its ability to make adaptive changes in the environment of reference (mutual influence).

In the 21st century the connection between the parts has gained heuristic significance (connectionism): to understand a complex system, relationships are just as important as the behaviour of the components. In fact, this analysis was used when Euler first formulated the "graph theory" to solve the problem of Könisberg bridges (1736); mechanisms and regularities typical of networks have been used to understand complex social systems, "real" and "virtual": circularity, speed and information management, power dynamics, resource exchanges, hubs, scale free law, redundancy and resilience, growth without global order are some of the most important aspects of a complex network and, therefore, of a complex system.

The consideration about complex systems has guided the entire survey on the social health and family situation linked to the issue of dementia in Brindisi social health district: the organization of the authority and the resources (private too) in the territory represent the close environment in which families with people affected by dementia operate; national and regional regulations (far environment) were important to compare the possible distance between these and the factual situation.

Far system and environment. Global and italian health situation

In 2012 WHO and ADI (Alzheimer's Disease International) detected an annual rate growth of 7.7 million new cases. The incidence of chronic-degenerative pathology has no geopolitical, economic and cultural boundaries, but there are considerable differences between countries in terms of social health interventions and legislative measures: in fact, only 8 out of 194 WHO Member States had a National Plan on dementia (WHO and ADI 2012). After four years, the global Alzheimer's report places emphasis on the quality of life of patients – Improving healthcare for people living with dementia. Coverage, quality and costs now and in the future (Alzheimer's Disease International 2016) – on the replies of the various territories and the organizational and community good practices. The urgency is given by current figures and

evaluations: dementia affects 47 million people worldwide and the figure is destined to triple by 2050, while welfare policies still have a secondary role. Moreover, only about half of the patients in high-income countries and one in ten in the middle and low-income countries received a diagnosis. Great emphasis is given to assistance: there are no clear "treatment pathways", in which roles and responsibilities are defined within the welfare system, setting standards to be monitored and respected. Instead, treatment pathways, structured and organized coordination, resources and continued assistance are common for people affected by other chronic diseases (diabetes, hypertension, cancer).

To carry out a comprehensive analysis of the phenomenon on a unitary basis, in December 2017 WHO launched the Global Dementia Observatory (Gdo), a web platform designed to provide easy access to the most important data and information on dementia in the Member States. Three areas are covered: health policies, service provision, information and research. The aim of Gdo is to provide the various countries with a support tool to measure the progress made in this sector, also in the light of what has been outlined in the Global action plan on the public health response to dementia 2017-2025: the traditional therapeutic models are now abstract and scarcely applicable to a reality that has diversified requests of health and care.

New concepts take over: Disease and Illness. The first one is meant to define the health problem in terms of physiological malfunction with reduction of physical abilities and life expectancy. Illness refers to the subjective experience of the disease, to the way in which a subject represents it and gives it a meaning; however, the subjective sense is also always socially determined (Cipolla, Giarelli and Altieri 2002). The Harvard Medical School adds a third concept, Sickness: it corresponds to the social description of the disease and therefore to the way in which a social group interprets and implements strategies related to the disease; behavioural and biological signs considered disturbing are converted into illness. The analysis of Sickness emphasizes how the medical facts are pre-constituted informally outside the clinical research environment (Maturo 2007). This conceptual intersection brings out the relational nature of health: the centrality attributed to the psycho-social connotation of it induces to elaborate a planning strategy which, starting from a participatory identification of needs, constructs aims and defines actions in an intersectional perspective; this in order to develop a strong interdependence between health services and other services in the area (Cipolla 2005). The flow of communication and resources will have to lead to a systemic pluralism and to the reduction of the typical separation between the health and social welfare system.

Organizational vacuum and regulatory delay created in Italy long distances between patient, healthcare network and institutions: even though innovative pushes have been proposed as well as the formulation of alternative solutions, redefinition of areas of relevance for the different roles, involvement and reconfiguration of skills, the answers in support of Alzheimer's are still lacking. Furthermore, despite the Dementia National Plan (2015), the progressive increase in the number of patients suffering from dementia and the lowering of the average age of onset (Istituto Superiore della Sanità – National Institute of Health – 2015), the national policy – and the regional policy in Puglia – has continued to reduce health and welfare spending without counteracting compensatory measures; thereby marginalizing patients and those who assist them.

In this regard, a strong North-Centre-South differentiation emerges with regard to social-welfare and health policies. In line with the contemporary local trends, the disease is narrowed by these dominant tendencies. Decentralization and local regulation of social policies are the product of a process started at the end of last century, that has disregarded the principle of universalism on which our National Health Service is built. Diseases such as Alzheimer's must be managed by the entire nation: access to and fruition of services can not be linked to local dynamics and variability of the limitations of the municipalities. For example, although Alzheimer's has been a reality for some time now, in our country it is still difficult to have an official census of updated data on the epidemiology; this happens for all mental illnesses, about which some shortcomings in

the information sources have been listed (Psychopathology Italian Society 2017):

1. Sources available from different authorities/institutions and various information not easily usable together.
2. Unsatisfactory information on supply, quality of services and public spending.
3. Absence of a longitudinal vision of individuals, essential to design policies and to assess their impact.
4. Integration and partnerships are also crucial to fully implement the Mental Health Action Plan of WHO which involves the statistics as well in several places.

The particularly alarming aspect concerns the heterogeneity in the field of ad hoc social and health policies. Also the "41st Annual Report on the Social Situation of the Country" (Censis 2007) detects a two-speed health care in the North and South: the distance between the two parts of the country has led to a worsening of the health situation among the citizens of the southern regions. The modification of the constitutional framework (State-Regions pact introduced by the Reform of Title V of the Constitution n.3/2001, ratified by the Stability Pact between the two orders) unequivocally contributed to increase the differences. The situation got worse ten years later (Censis 2017) with the growth of the family spending on health and the North-South gap concerning the satisfaction about health in different regions.

The same outcome emerges from the results on non-self-sufficiency: while the needs increased, the state funds were cancelled in 2012, 275 million were allocated in 2013 for non self-sufficiency, but it was 60% less than in 2008 (Non Self Sufficiency Network 2013). Two years later Censis (2015) noted that to deal with the private cost of assistance to non self-sufficient people, 910,000 families had to "tax" themselves and 561,000 families spent all the savings and/or had to sell their house and/or got into debt. The last data (Non Self Sufficiency Network 2017/18) shows a worse situation: declining trend in the financing of various types of services (residences, home assistance, economic support), prevalence of cash transfers on services (55% versus 45%), ideas regarding the need to lower the quality of services, perennial

uncertainty about the dichotomy coverage/intensity. Furthermore, there is an increase in the need of families for help in informative "paths" and consultancy about activities that are often sacrificed for budgetary reasons: health, contributions, aids, facilities at work, caregivers, barriers, disability, etc...

To further understand the process of interventions on dementia, the various National Health Plans still remained in ambiguity when they treated Alzheimer's, generically including it in the indefinite category of mental health although years before the civil society had committed to define categories and strategies of action. In 1999 the Chart of Patient's Rights has been drafted by Italy Alzheimer's Federation and published in the European Handbook: the cornerstone of the declaration concerns safeguarding the quality of life of the patients and of those who assist them.

Italy Alzheimer's Federation (1993) and Italian Alzheimer's Association (1985) have concretely contributed to raising awareness about the issue related to dementia and the effects on family and caregivers, through analysis of the situation, consultancy with national and supranational government authorities, sharing knowledge among citizens and health workers and lastly information-assistance to patients and their families. As for the creation of protection and therapy networks for patients, the achievement has been complex and frustrating, given the poorly associative culture of Italians, as always underlined by Istat report.

Within this situation, an important institutional change is represented by Progetto Cronos (2000), the largest clinical-epidemiological study carried out in Europe on Alzheimer's disease. The nation-wide observational study was possible on the basis of a network of specialistic centres, UVA (Alzheimer's Assessment Units), in collaboration with general practitioners and pharmacists, thus ensuring care continuity between hospitals and local assistance. The aims were exclusively of medical-pharmaceutical nature, including patient acquaintance, and little or nothing worked in the information-assistance system aimed at families and caregivers.

Extension of Progetto Cronos is Commissione Alzheimer (D.M. 22 February 2002), established for the purpose of developing an accu-

rate knowledge of the health needs of the population affected by Alzheimer's and the social consequences of the disease; of fulfilling a range of functions, including coordination actions in the regions through the presentation of consultative, study and proposal analysis. However, from a national survey on the characteristics of UVA, Day Centers, Nursing Homes, Integrated Home Care carried out by Centro nazionale di epidemiologia, sorveglianza e promozione della salute (National Institute of Health) discomforting results emerged, as they followed those of 2006 (Faiella 2015).

A crucial step forward has been done – as mentioned above – with the Dementia National Plan (Gazzetta Ufficiale, n. 9/13-01-2015). The critical issues highlighted in the Plan are not new: services which are not integrated (diagnosis, rehabilitation, welfare); specialistic centres (Alzheimer Assessment Units) established without need-based programming and with a strong disparity in composition and function; lastly, after 14 years Alzheimer Assessment Units remain the main access point for patients with dementia and have in the meantime been transformed into permanent services variously named in some regions, so it has become necessary to start a process of unification of these services. It is necessary to tackle these criticalities through 4 aims: 1. interventions and measures of social and health policy; 2. creation of an integrated network for dementia and implementation of an integrated management; 3. implementation of strategies and interventions for the appropriateness of therapy; 4. increase of awareness and reduction of social stigma to improve the quality of life.

The National Institute of Health had immediately started to monitor some key aspects related to the implementation of the National Dementia Plan by the Regions: the health system related to dementia is not very efficient and organized, rather local and very focused on drug therapy and less on assistance. Above all, there are no figures or structures capable of activating assistance-help-advice services to the family members; lastly, there is a cognitive gap for the constant monitoring of the general situation and specific cases, which are also preparatory for the activation of ad hoc interventions. Much is being done on the cultural and terminological level to train social health workers and

citizens, thus avoiding situations of stigma and social and institutional exclusion, still rather widespread and determining the poor quality of life of patients and family members.

Close system and environment. Health situation in Brindisi

The survey carried out by the researchers of the Salento University aims to investigate the complexity of the socio-structural and relational dimensions that characterize the phenomenon of dementia in the city of Brindisi, first of all by providing a first mapping of the services (public and private) specifically addressed to the disease; identifying also the needs of the families of patients with dementia/Alzheimer's; lastly, verifying the role of associations as support for families and institutions.

The research – first detection of the phenomenon in Brindisi – is composed of 4 phases. The first phase concerns the analysis of data from the institutions (Osservatorio provinciale sulle politiche sociali, Unità di Valutazione Alzheimer, Centro Salute Mentale). A brief overall assessment concerns the organization and storage of information: the computerization and the standardization of data were absent, making it difficult to find information and extending the time needed to consult the data. In this regard, the information provided in the sections dedicated to the structures and social-health services in the province of Brindisi in sanita.puglia.it website was empty or insignificant. Secondly, information about users with issues related to dementia was still in a paper archive. Both archives consulted were necessary to detect and analyse the extent of the phenomenon in the city of Brindisi and compare it with the rest of the province and with Italy. Furthermore, user lists and their characteristics were useful for constructing a stratified probabilistic sampling. It was not possible to do what was planned because it was only possible to access the data of the Mental Health Centre (CSM). It is striking the uncertainty of the diagnosis which does not allow an exact placement of the user in the health archives. This has an effect on internal communication within the Local Health Authority and external relations between the Local Health Authority and other public institutions with duties related to welfare services. Another problem related to communication, this time

between Local Health Authority and users, is the absence of some telephone numbers of the patients, almost always the only way to have a quick contact.

The second phase involved social health structures (presence and type): the information was collected from the archives and from interviews with the managers in charge of the offices. The creation of the first social health structures in Puglia dates back to 2007 (Regolamento Regionale n. 4/2007, application of Legge Regionale 19/2006). The legislative gap, currently still very marked in terms of specific structures for people with dementia/Alzheimer's, is partly filled with the art. 60ter of Regolamento Regionale 7/2010, which regulates the institution of Day Centres specific for dementia/Alzheimer's, but for individuals who do not have severe motor deficit and can be managed through semi-residential treatments. A gap remains in the field of ad hoc residential facilities, conceived to welcome the patient with Alzheimer's and the therapy needs. A defect to which must be added another limit concerning the institution itself of the structures that, as provided for L.R. 19/2006, is entrusted to "management experimentation". To try to compensate for such a regulatory vacuum, Alzheimer's patients over 65 years old can be welcomed in Residential Homes which foresee – as prescribed by the Regional Regulation 4/2007 – that there may also be people with dementia/Alzheimer's under the age of 65, provided they do not need complex health services. There is also a type of extra-hospital structure with high health intensity, the Nursing Home (D.G.R. 210 R.R. 8/2002), which includes specific residential modules for people with Alzheimer's.

A rather lacking picture emerges in several respects. The critical issues are evident on several sides and offer a discouraging glimpse of the territorial situation examined in the two-year period 2012-13. Among the multiplicity of the problematic aspects connected to the phenomenon, the absence on the territory of ad hoc residential structures constituted (and constitutes) the biggest limit (Centro per il controllo e la prevenzione delle malattie del ministero della Salute 2017). An additional load in terms of management derives from the coexistence of subjects suffering from different pathologies:

this can be a source of discomfort for the guests, as well as a physiological disorganized and sometimes confused work for the operators. This situation puts a strain on the caregivers' ability to orientate in the articulated universe of care, as well as the ability and patience to combine and calibrate the efforts alongside those of specialistic figures, especially in relation to the steps that mark the degenerative process.

This dimension emerges from the other phases: the survey on the management of the patient; case studies about the experience of some caregivers; a survey based on privileged witnesses (doctors).

For what concerns the survey only 58 caregivers have been reached, living in Brindisi, out of 210 listed in the archives of Centro di Salute Mentale (tab. 1). This figure has been increased up to 76 individuals, by contacting subjects living in other municipalities through the collaboration of some general practitioners.

Tab. 1 Overview of contacts with caregivers for the sample survey

Relationship between number of cases and subjects interviewed	Total number of cases	210
	Questionnaires administered	58
	% of success	28%
Causes of missed interviews through questionnaire	**Refusals**	
	For disinterest	23
	For psychological status of the caregiver	51
	For wrong diagnosis	12
	Phone numbers	
	Inactive	33
	Non existent	13
	No answer on the phone	12
	Missed appointments without apparent reason	8

From the archives of the Mental Health Centre in Brindisi

The aim was to identify the emerging needs of the people with dementia/Alzheimer's and of caregivers.
The areas of the questionnaire were:
1. level of pathology and therapy needs;
2. caregivers and work-life balance;
3. social capital of reference;
4. role of institutions, in particular Municipality, local health unit (Asl) and general practitioners;
5. general assessment of services by the respondents.

Most of the sample – composed of 29 men and 47 women with an average age of about 59 (minimum age 35 years, maximum age 83) – is included in 45-50 and 60-65. Most of them are sons of the patients (56%), followed by spouse (28%), daughters/son-in-law (9%), brothers/sisters (4%) and 3% declared to be nephew or other. 92% of the sample lived with the patient. Generally caregivers were over 60 and women (wife, daughter, daughter-in-law).
The analysis of the data clearly shows how the disease afflicts not only the patients but also the family members and those who assist them, so that caregiver and patient become a single system of action. Caregivers claimed to dedicate to patients more than 16 hours a day, keeping for themselves very few hours a week. Facing the progress of the disease, they felt more and more helpless and this feeling was exasperated by the awareness of the absence of effective drugs, by effort and stress in the day-to-day management of the patient and by the frustration in not seeing recognized their effort. Lastly, the deep sense of loneliness derived from the inadequacy of parental and institutional supports (public and private), also in the form of information on the approach to the patient and on the territorial resources. This loneliness increases gradually with the progress of the disease, which becomes a disruptor first on a psychological and physical level, then on a social and economic one: with the collapse of the psychic faculties, the patient is forced to abandon all daily activities, including work, with the risk of irreversible impoverishment; while the caregivers, especially if alone, can not bear the emotional and time load of the situation: in fact, both for emotional collapse and for lack of time they are absorbed by the needs of the patient, who tears them away from relational and social life. Free time disappears for almost all respondents and almost everyone has complained about the burn out situation: psychophysical fatigue, muscle aches, headaches, low or alternating mood, insomnia and eating disorders were the reasons listed and that constitute a deterrent to the enjoyment of leisure moments. Lastly, the respondents found great difficulties at each stage of the patient's illness: at first incompetence in dealing with the disease and approaching health facilities (disorienta-

tion), which is witnessed by the doctors interviewed; later with the advancement of mental and behavioural deficits, for which the caregivers often do not find informal and formal-institutional supports (isolation and mistrust). Thus assistant and assisted live in symbiosis: the deconstruction of the personality of the second is counterbalanced by the identity decline of the first; s/he, especially if alone, begins to interpret the self according to her/his role as "nurse": by changing her/his environment (illness of the family member), s/he changes the identity system (caregiver and no longer worker/partner/friend/etc.) and this in turn modifies the environment itself (made of care needs, health care assistants and facilities).

The current society is increasingly based on productive efficiency (Giddens 1990) rather than on the enhancement of social relations, especially intimate, now mostly aimed at achieving exogenous purposes such as prestige, money and power (Fromm 1976). This structural and cultural situation determines in psychic systems (individuals) an ability of action characterized by rationality, which develops attitudes not aimed at a deep knowledge of others and the creation of solid bonds (Bauman 2003).

This brief digression introduces one of the elements that contribute to the disorientation and solitude of the "assistant-assisted" system (Bauman 2006) and emerged from the interview with the general practitioners: also the doctor-patient relationship is based on the efficiency, on time and patience in examining the changes experienced by the patient through a fruitful dialogue. General practitioners are the first and fundamental step for an adequate health care path, but often they do not know how to recognize the first signs of dementia/Alzheimer's and not few times several years pass before the diagnosis is recognized.

From the in-depth interviews to doctors and caregivers, another topic emerges, which is an effect of our social and cultural system: the lack of a) an informal community, made up of relatives, friends and neighbours and b) an institutional community, represented by an adequate health organization and by sufficient territorial structures suitable to welcome/assist the patient, enhancing the individual.

Caregivers need concrete references, guides and specialists to turn to in order to understand how to deal with everyday life and relieve it from the burden of the management; they express the need to have real assistance in their territory, possibly a dedicated medical coverage that can break down waiting lists and guide caregivers and family members on obtaining financial aid and information on social-health care. To this situation it must be added the lack of associations, whose significant presence would instead compensate for some shortcomings related to material, informative and moral support.

Conclusion.
A new health humanism from the observation of complex systems

The 18th National Congress (2014) of Collegio dei Primari Oncologi Medici Ospedalieri (Association of Oncology Head Physicians) clearly stated its vision in the title: "Complexity management in Oncology: 'swarm intelligence'". The Congress invited to abandon a top down and "monocratic" approach to follow a flexible organisational model, that is a complex model suitable and consistent with the unpredictable and chaotic resources at stake. Until then workers were considered simply production tools functional for the organisation, and the head physicians were supposed to check the external uncertainty through a frantic research of a simplifying rationality. Later it was intended to adopt new organizational dynamics characterized by adaptability, diversification, designed to enhance the subjects involved in their diversity, integrating roles, making communication flow between the different organizational levels, also in order to allow a prompt internal change and an efficient openness to technical, technological and strategic innovation. This happens because the environment – represented by patients, people and organisations of different kind – is mutable, turbulent, uncertain. This does not mean choosing a managerial anarchy, but an organisation whose vertexes simplify and foster bottom-up solutions, promote collective intelligence, starting a process of amplification of power and allowing an efficient interconnection among the internal levels and among them and the environment.

The focus of the leadership should be to delegate tasks to foster the development of distributed intelligence in organizations, that is, the

skills of human being in networks. This is crucial for the issue of health and welfare management of people affected by Alzheimer's and their caregivers: self-organisation is reached 1) promoting the creation of a network within the company, where the hub is the intelligence of people and the connections are represented by different interactions among them and through 2) an external network, involving all the potential relevant stakeholders from the private sector too and not directly linked to healthcare activities (computer scientists, architects, sociologists...), in an interdisciplinary view.

The initial aim is to adopt a simple strategy to generate complexity; the opposite of the classical vision, which starting with complicated rules generates simple behaviours (Ashmos et al. 2002). The simple strategy of participation consists in connecting people to each other, leaving them autonomous at the same time with control mechanisms and small procedures.

To remedy the abandonment of caregivers and patients for what concerns dementia, it is necessary to re-establish a trust relation with the territory and to build spaces of profitable interconnections. Classical science and modern rationality outdistanced organizations and their territory – conceived as a synthesis of space, history, culture and relationships – leading us towards a constellation of non-communicating fiefdoms. We now understand that the territory is a place of distributed intelligence, because it constitutes a field of relational and communicative resources, capable of integrating thousands of decentralized and interdependent intelligences: when these interact with each other, they produce organized and efficient behaviours (Quadrio Curzio and Fortis 2002).

In order to reach this ambitious goal, a new organisational humanism is required, a culture of participation and first of all, a culture of continuing training, not only related to professional contents. Indeed, it is necessary to have humanistic attitude and inclination able to foster a wise use of techniques and technologies and to overcome a general relational and communicative illiteracy.

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The Right to Health in a multi-level perspective

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A fundamental right of every human being is the right to health, which implies the right to life and as such, it is fundamental, unavailable, non-erodible and practically enforceable. The right to health is a universal principle that emerges in the Preamble of the Constitution of the World Health Organization (WHO); it is universally recognized by the human person, and directly ascribable to the supreme value of life, which constitutes the capacity for immanent action (Sgreccia, Spagnolo 1999).

In accomplished democracies, the right to health has an absolute value. This absoluteness is due to the principle of being recognized by everybody as well as from the fact that it guarantees an *erga omnes* value, meaning that nobody can assume facts or behaviors that could harm such a right.

For instance, article 32 of the Italian Constitution defines the Right to health as a fundamental[1] and absolute right, protected by the Constitution in a full and unconditional way with respect to everyone, without distinction of race, religion, political belief, economic and social conditions, therefore a "precondition" for the exercise of all other rights (Luciani 2003).

In accordance with the Italian Constitution, in fact, the need to protect physical health is emphasized as much as the psychical one. In particular, the Constitutional Court focuses on *health care[2]*, i. e. all the "*positive interventions for the protection and promotion of human health*", which requires not only care but also an affective and relational support. It is then clear that an ex-

haustive analysis of the dynamics and problems connected with the "right to health" issue cannot be separated from the existing European legislation in this field, given the complementary role that this legislation has in the Member States' legal systems (Cuocolo 2005).

Since from the initial foundation of the Community, it is not possible to elicit a comprehensive discipline of social rights. The failure to include a comprehensive set of social rights in the Community texts is due to the historical postwar situation in Europe, characterized by the government control of monetary policy, which is crucial for the construction of the State system. However, it is possible to find a track of social measures from the founding Treaties and, above all, relating to health care. In particular, article 100A of the treaty establishing the European Community provided that the Commission should pursue a high level of public health protection when adopting measures aimed at reconciling Member States' legislation.

An important step forward was made in 1986, with the Single European Act, which combines the three Communities with the European political cooperation and extends the Community's competences concerning the subject under discussion (Cartabia, Weiler 2000). The Single Act adds a Title on economic and social cohesion to the EC Treaty (article 130); it also highlights the importance of the European Community to pursuing social justice objectives in the Preamble, recalling the principles of the Social Charter of the Council of Europe approved in 1961 (D'Intino *et alii* 2006, 7-9).

However, these predictions cannot be translated into a catalogue of fundamental social rights. If, therefore, emphasis must be focused on launching the development of Community social policies, it is not possible to identify a real breakthrough in the Single European Act in terms of protecting the rights of individuals and

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[1] In article 32, the Constituent stated that «*Republic protects health as a fundamental right of the individual and in the interest of the community, and guarantees free treatment for the needy. No one can be obliged to take any particular medical treatment except by legal provision. Under no circumstances can the law violate the limits imposed by respect for the human person*».

[2] Constitutional Court, judgment of 30th September 1999, n. 382, in *Giurisprudenza Costituzionale*, 1999.

explicitly in health protection, which must be pursued in all Community development policies.

In 1992, the Treaty on European Union (TEU) known as Maastricht Treaty amended the TEC by identifying the achievement of a high level of health protection as one of the Union's main aims. Title X of the TEU is dedicated to *"Public Health"* and it is composed of a single article 129; it underlines the need for Community intervention to complement State action in order to achieve a high level of protection of human health (Pitino 2003, D'Intino *et alii* 2006 , 162).

Finally, article 6 of the TEU, in line with the approach developed by the Court of Justice, affirms the Union's obligation to respect fundamental rights guaranteed by the European Convention on Human Rights as they result from the traditions common to the Member States as general principles of Community law (Ferrari 2001, 4).

However, this provision does not seem to be extendable to social rights: the reference made by article 6 in fact, it is apparently restricted to the fundamental rights of freedoms, which are effectively governed by the above-mentioned Convention. The rights set out in the European Social Charter and, therefore the right to health are thus excluded.

In the framework set out in the Maastricht Treaty, it should be pointed out that the principle of harmonization of national policies has been tending to be replaced by the open method of coordination, which aims to share information between Member States in order to identify best practice in individual areas, without imposing minimum requirements for individual states

The real breakthrough in the protection of social rights came in 1997 with the Amsterdam Treaty, which entered into force on 1st May 1999 (Zanetta 2003, 48).

Article 3 (p) requires Community action to contribute to the attainment of a high level of health protection. The most relevant aspect consists in the introduction of the article 152 TEC, replacing the former Article 129 TEU, which enshrines the basic principles of Community action on the protection of human health. More exhaustively, article 152 (1) provides that a high level of health protection is to be ensured in the definition and implementation of all Community policies and activities. This Article provides that Community action is to be directed primarily towards the prevention of diseases by encouraging research to their causes and transmission, as well as health information and education. It is clear that the provision introduced by the Treaty of Amsterdam differs from Article 129, which provided for a very marginal role for the Community institutions, merely as a subsidy for the action of the Member States. On the contrary, article 152 places health protection as a general criterion to be followed in the implementation of all Community policies. The protection of health is therefore not a material matter, but a criterion, a Community principle in the light of which the action of the Community bodies can be read. It is of fundamental importance to clarify that in Community law, after Amsterdam, the protection of human health is almost an obligatory result, which cannot be sacrificed in balance with the other guiding principles of Community action.

Moreover, Article 152 (1) takes into consideration not only the "healing" aspect of health protection, but also the preventive aspect; this is, in particular, a profile that considers the Community provisions more advanced than those of the national Constitutions, including the provision of Article 32 of the Italian Constitution and that requires new standards of protection in member states, also with respect to prevention aspects.

Article 152 (2) provides that "Community shall encourage cooperation between Member States in the areas covered by this article and, where necessary, support their action. The Member States in liaison with the Commission shall coordinate among themselves their policies and programs in the areas referred to in paragraph 1. The Commission, in strict contact with the Member States, may take any useful initiative to promote such coordination". The provision incorporates some of the principles contained in above-mentioned article 129, that reiterates the necessary harmonization of Member States' policies.

Article 152 (3), supplementing the principle of harmonization set out in the second subparagraph, provides that "The Community and its Member States foster the cooperation with third countries and the competent international

organizations in the field of public health". The achievement of the objectives set out in Article 152 may require Community measures to complement the actions of Member States, but it mainly involves encouraging cooperation between States, in accordance with the principle of subsidiarity.

At institutional level, defined in Article 152, incentive actions shall be adopted by the Council acting in accordance with the co-decision procedure, while recommendations shall be adopted by qualified majority on a proposal from the Commission. The Council, therefore, becomes one of the main players in achieving health protection standards. Nevertheless, the provisions contained in the fourth subparagraph are not exhaustive of Community action in the field of health, but are only part of Community competence; this is also confirmed in the open clause of point (c), which requires the Council to adopt any incentive measure, provided that it does not involve forced harmonization of national legislation (Pitino 2003, 306).

One of the most important forecasts introduced by the Amsterdam Treaty is that of Article 152 (5), according to which: "Community action in the field of public health fully respects the responsibilities of the Member States for the organization and delivery of health services and medical care"; direct Community intervention in the provision of health services is therefore excluded. The fifth subparagraph, combined with the impossibility for the Council to adopt harmonization measures, leads to the conclusion that there is still a lack of instruments for regulating the public health service at Community level, the definition of which is left to the legislation of individual Member States, which often differ widely from one another (Cilione 2003, 75).

It is not easy to balance the actual scope of the statements of principle contained in the original Community law: while the guarantee of high levels of health protection is a fundamental principle of all Community policies, on the other hand, the community lacks the means to regulate the forms of health protection in practice, that must be guaranteed to all European citizens (Sciullo 2004).

These considerations raise a few questions about the content of European citizenship, which does not seem to identify itself with a set of Community social rights yet.

The final step on the road to European social rights before reaching the Constitutional Treaty is the Charter of Fundamental Rights of the European Union, which was adopted in Nice in December 2000. One of the characteristics of the Nice Charter is the particular system, which, although it acknowledges social rights, does not divide them according to traditional generations.

In this regard, it should also be pointed out that the Nice Charter, although it has a high symbolic value as a result of a solemn proclamation, has no binding force in terms of positive law. The principles of the Nice Charter are useful mainly as interpretative arguments and that has a particular significance in consideration of what it is expressed by the article 6 of the Maastricht Treaty, which sets out a circular dynamic of fundamental rights (Azzena 2001, 135).

However, it is not possible to compare the extent of a legally binding act with that of an interpretative instrument, particularly in relation to social rights, which, although largely governed by the Charter, are not covered by the article 6 TCE.

The Charter's Preamble strengthens the weight of fundamental rights in the Community system as they derive from constitutional traditions and international obligations common to the Member States, the TEU, the Community Treaties, the European Convention for the Protection of Human Rights and Fundamental Freedoms, and the Social Charters adopted by the Community as well as the Council of Europe. In addition, the rights recognized by the jurisprudence of the European Court of Justice and the European Court of Human Rights are reaffirmed. The provisions concerning the right to health protection are the first three articles included in Chapter I, "*Dignity*".

In particular, Article 1 recognizes human dignity as inviolable, prescribing the necessary respect and protection; Article 2 guarantees the right to life for every individual; Article 3, finally, affirms the right to physical integrity, dictating specific prescriptions for medical activities. These provisions are undoubtedly important and clearly demonstrate the protection afforded by Community law to aspects of the negative right to health, i. e. the right not to receive

harm to one's own health. As far as the positive aspects of health protection are concerned, the analysis must focus on Article 35 dedicated to the" Protection of health" in Chapter IV dedicated to" Solidarity".

Article 35 provides that" Everyone has the right of access to health prevention and medical treatment under the conditions laid down by national laws and practices. A high level of human health protection shall be ensured in the definition and implementation of all Union policies and activities". While the second period of the provision reproduces Article 152 of the Treaty of Amsterdam, the first period introduces even more explicitly the right to preventive and rehabilitative treatment to protect individual health.

Despite the limited legal scope of the Nice Charter, it is essential to note the definitive separation between the concept of the protection of human health and the pursuit of other Community objectives and the qualification of the right to health protection as a right of the individual. On the other hand, the wording used in the Nice Charter does not serve as a basis for Community competence in regulating the public health service; The Charter states that the right to prevention and treatment is guaranteed within the limits of national laws and practices.

In a certain sense, this provision is the result of an obligatory line of action since, from the Preamble onwards, it is detected the scope of the rights set out above all in Article 51, which states that the Charter" does not introduce new powers or tasks for the Community and the Union, nor does it modify the powers and tasks defined in the Treaties".

It is crucial at this point to reflect on the effective protection of the law at Community level. In this sense, first of all, a negative limit can be identified, which consists in the choice to refer to the laws of the member states the organization and the concrete regulation of the public health service; a consideration resulting from the application of the principle of vertical subsidiarity that requires to allocate functions to the level of government as close as possible to the citizens (Arena 2006; Albanese, Marzuoli 2003; Moscarini 2003; D'Intino 2006).

This choice is due, on the one hand, to the different traditions of social rights in the various European experiences, and, on the other hand, to the numerous and fragmented forms of regulation which are based on different models and which provide for different forms of division of competences between the state and decentralized levels of government (Cuocolo 2005).

It can be said, therefore, that the competences of the health service are still destined to be placed at state or sub-state level for a long time, as it is indirectly confirmed in the *Green Paper on services of general interest* (COM-2003-270), which attracts only those public services of economic importance to the Community competition rules. However, this does not detract from the fact that the Community institutions can also play a leading role in terms of health protection. In order to understand the extent to which this is happening, it is necessary to wonder what instruments and techniques for regulating the right to health are permitted by the Treaty that establishes a Constitution for Europe, approved by the Intergovernmental Conference on 18 June 2004 in Brussels and signed on 29 October 2004 in Rome (Letta 2006, 14-15). The Constitutional Treaty in Part II includes the Charter of Fundamental Rights and, in particular, in article II-35 it takes over article 35 of the Charter, which deals with the protection of human health.

On the other hand, article 152 TEC, introduced by the Treaty of Amsterdam, is reproduced in article III-179 within Section I" Public Health" where Chapter V is dedicated to "Sectors in which the Union may decide to demonstrate coordination, integration and support action".

In the adopted perspective, it is relevant the wording of the seventh paragraph corresponding to article 152 (5) TEC. In fact, it is well affirmed the competence of each member state to define public health policies and the organization and delivery of the public health service; it specifies that "the responsibilities of member states include the management of health services and medical care as well as the allocation of resources destined to them".

It is evident that Community action in the healthcare sector would never lead to the creation of a European health system. It can be assumed that human health is not a competence of the Union, but only a criterion to be followed in regulating subjects. In support of this

argument, it could be emphasized that the Nice Charter did not recognize a subjective right to health protection. If this were the case, health protection would be a sort of" filter" through which to read every Community act, but not a competence of the Union that would legitimize the development of autonomous policies.

On the contrary, it can be assumed that health protection, even with all the limitations highlighted, is a proper subject that should belong to the shared competence of the Union (Pitino 2003).

This reconstruction is confirmed by article I-13 of the Constitutional Treaty that identifies the areas of shared competence of the Union in point (b) of the second paragraph; it makes express reference to social policy, as regards the aspects defined in Part III. Where this is true, the guarantee of a high level of human health protection can also be the subject of specific policies and, in this context, the provision of specific" essential Community levels" of health care could even be envisaged.

Community protection could thus absorb the constitutional principles of the state and, as far as Italian law is concerned, the essential levels of benefits relating to civil and social rights that must be guaranteed throughout Italy. The scenario is certainly stimulating and it is not excluded that it may have significant developments in the future (Morbidelli 2004, Cuocolo 2005).

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