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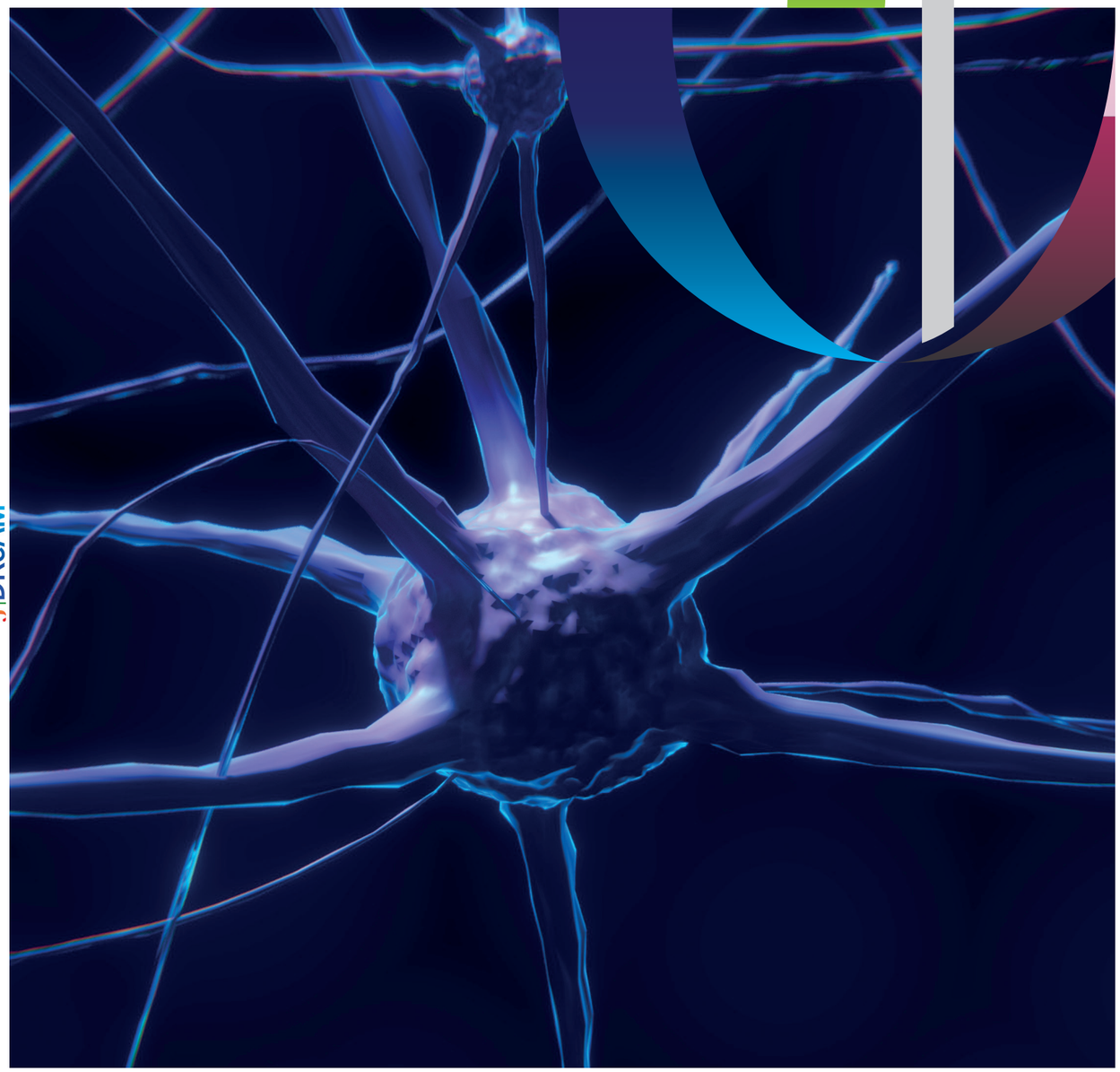

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laboratorio diffuso di ricerca interdisciplinare applicata alla medicina

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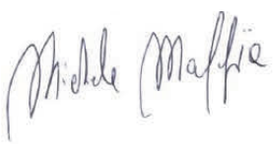
Editor in Chief



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OMICS Sciences: toward omics personalized medicine

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Abstract
The omics sciences of systems biology including genomics, transcriptomics, lipidomics, metabolomics, and proteomics, aim at understanding the biological mechanisms that give rise to the phenotype of an organism by using high-throughput technologies with the promise of great medical advances. The importance of all these sciences is that all, with the exception of genomics are context dependent. Genome is constant in time and place in each cell of an organism, but the entire complement of messenger RNA molecules, proteins and metabolites in a cell, tissue, organ or organism varies with physiological, pathological or developmental conditions (Keusch 2006). The term "omics" represents the study of biological processes as systems. It deciphers the dynamic interactions between the numerous components of a biological system to analyse networks, pathways, and interactive relations that exist among them, such as genes, transcripts, proteins, metabolites, and cells. This new scientific vision has opened the way to new research strategies and experimental technologies that have transformed the study of virtually all life processes. Expansion of the "–ome" concept was incessant and has created a host of new terms, including bacteriome, cardiome, epigenome, erythrome, immunome, microbiome, neurome, connectome, osteome, physiome, proteinome, transportome, degradome, psychome, transcriptome, and many others.
In the present review, these concepts are briefly introduced with a major focus towards proteomics.

Keywords: omics sciences, proteomics, bioinformatics, personalized medicine

1. Genomics, metabolomics, lipidomics, epigenomics

Genomics is the study of the genomes of organisms. For several years genomics was at the forefront of omics sciences, we were in the "Genomic Era". Because many diseases are intimately associated with genetic mutations, the idea that the solutions for human pathologies lie on genes has catalysed the interest of scientists for years, making genome-based analysis methods a central approach in omics science and setting the scene for the completion of the Human Genome Project (HGP), undoubtedly a major landmark event in the field of genomics after the discovery of the double-helical structure of DNA. Since the completion of the human genome project, our ability to explore genome function is increased in specificity. In fact, substantial changes have occurred in the study of genome owing to the introduction of several approaches to DNA sequencing and expression. The massive quantification of messenger RNA (mRNA), genome copy number, and single nucleotide polymorphisms (SNPs) by microarray technology has enabled to assess the expression of tens of thousands of genes shedding light on the mechanisms underlying human pathologies, providing the basis for stratifying patients and predicting outcomes in a variety of diseases. Together with microarrays, recent advances in DNA sequencing with the introduction of next-generation sequencing (NGS) technologies have made possible unprecedented extensive analyses of genome of

individuals. Presently, there are three main NGS systems: the Roche/454 FLX, the Illumina/Solexa Genome Analyzer, and the Applied Biosystems SOLiDTM System. Each one, by a different approach, seeks to amplify single strands of a fragment library and perform sequencing reactions on the amplified strands. Together with these technologies, a new generation of single-molecule sequencing technologies is now emerging offering advantages over current sequencing methods including small amounts of starting material (theoretically only a single molecule may be required for sequencing), and low cost.

An important consequence of this new emerging scenario was the creation of multidisciplinary teams and the formation of large-scale collaborative networks to handle and integrate these large amounts of data. The HGP was the first example of a large collaborative project; others include the Cancer Genome Atlas (TCGA) and the 1000 genome project. TCGA has achieved comprehensive sequencing, characterization, and analysis of the genomic changes of major types of human cancers providing also a platform for researchers to search, download, and analyse data sets generated by TCGA (http://cancergenome.nih.gov). The 1000 genome project aims to establish an extensive catalogue of human variations from 25 populations (www.1000genomes.org). The project provides an international open access resource that serves as a basis for subsequent phenotype related studies (www.1000genomes.org).

Endogenous metabolites can be seen as part of the downstream output of the genome, complementary as "upstream" changes in genes (Spratlin, Serkova, and Eckhardt 2009), and their study together with exogenous metabolite is important to understand what happens in an organism. The term "metabolome" was introduced in 1998 as the total metabolite content of a biological sample, an enormous complex and dynamic number of components that belong to a wide variety of compound classes, including nucleic acids, amino acids, sugars, and lipids.

The term metabolomics was used for the first time in 2000 by Fien, to describe the discipline devot

ed to the study of global metabolite profiles produced in biosynthetic and catabolic pathways from biological systems or originating from host-specific microbes and the intake of nutrients and pharmaceuticals, present in cells, tissues, and biofluids.

Different techniques have been developed to investigate the metabolome, distinguishing the different metabolites on the basis of their chemical and physical properties (Cacciatore and Loda 2015).

Most commonly used techniques for metabolomics are nuclear magnetic resonance (NMR), gas chromatography coupled to mass spectrometer (GC–MS) and mass spectroscopy (MS). Recently published papers describe the application of metabolomics in the study of heart disease, cancer, and other human pathologies. Another potential application of metabolomics involves the definition of biochemical pathways that contribute to drug response. Pharmaco-metabolomic signatures have also been identified for several drugs to predict individual responses to broader medical, dietary, microbiological or physiological challenges.

Data generated experimentally by metabolomics are available in metabolite databases such as the Human Metabolome Database (HMDB). HMDB (www.hmdb.ca), the equivalent of the Human Genome Project for metabolomics, is a resource dedicated to providing scientists with the most current and comprehensive coverage of the human metabolome. Created in 2004, it contains information on biological properties, ontology, spectra and physical properties of human metabolites as well as data regarding metabolite concentrations, disease associations and tissue locations. Started as an inventory of 2500 small molecules, its content has increased to 15000 entries (Liesenfeld et al. 2013).

Lipidomics, term emerged for the first time in 2003, is a sub-discipline of metabolomics that aims to define all of the lipid molecular species in a cell, including the metabolizing enzymes and lipid transporters and understand how lipids function in a biological system. In detail, lipidomics involves the identification of individual cellular lipid species, including the type and number of individual atoms in each lipid species, and their stereoelectronic interactions with other lipids and proteins. Cells use 5% of their genes to synthesize their lipids that fulfil three main functions. Lipids not only forms the bilayer matrix, not only are used as energy stor-

age, but can also act as second messengers and participate in signalling via specialized microdomains, lipid rafts, that have large amounts of lipids. The field of lipidomics is rapidly growing as demonstrated by the great utility of this approach to improve diagnostic–prognostic capabilities for human disorders, and for the identification of new classes of lipids. Similarly to what happened for genome and metabolome, an attempt to characterize the mammalian lipidome has started under the LIPID MAPS initiative (http://www.lipidmaps.org) in 2005. To accomplish this goal, a consortium of twelve independent laboratories from seven academic institutions and one company has been formed (Schmelzer et al. 2007; Fahy et al. 2009).
Early separation and identification of lipids started with GC and HPLC, but other technologies coupled to chromatographic methods, such as MS, Matrix-assisted laser desorption-ionization/time of flight (MALDI/TOF), NMR, and quadrupole–linear ion trap (QTRAP), provide now a powerful approach to the global analysis of complex lipid mixtures. Given the enormous complexity of cellular lipidomics, it has been estimated to encompass around 180 000 – 200 000 different lipid species, high-throughput technologies are needed to approach the entire lipidome of cells. MALDI can also be used to reveal the distribution of lipids in tissues with the technique of imaging mass spectrometry (IMS) obtaining information relevant to the local distribution of lipids as they occur in tissues.
The term "epigenetics" was originally coined by Conrad Waddington to describe heritable changes in a cellular phenotype that were independent of alterations in the DNA sequence. DNA methylation, the transfer of a methyl moiety from S-adenosylmethionine (SAM) to the 5-position of cytosines in certain CpG dinucleotides, represents the most studied of epigenetic processes with a great impact on gene expression. Evidence is mounting for a direct link between DNA methylation and human diseases. Chromatin changes are another central epigenetic process with a role in transcription, repair, replication, and condensation. Overall, there are now at least four different DNA modifications and 16 classes of histone modifications. Gene-specific techniques for determining DNA methylation include bisulfite sequencing, methylation-specific PCR (MSP) and quantitative MSP. The coupling of NGS platforms with established chromatin techniques such as chromatin immunoprecipitation (ChIP-Seq) represents the standard for identifying binding site locations for individual protein and histone modifications.

2. The post-genome era: Proteomics

The complete characterization of all proteins has been the goal of proteomics since its inception more than 20 years ago. Originally coined by Wilkins et al. in 1996, the term "proteome" refers to the entire PROTEin complement expressed by a genOME (Abdallah et al. 2012).
Proteomics techniques offer several advantages over genome-based technologies, as they directly deal with the functional molecules rather than genetic code or mRNA abundance. Even though there is only one definitive genome in an organism, it codes for multiple proteomes since the accumulation of a protein changes in relation to the environment and is the result of a combination of transcription, translation, protein turnover, and posttranslational modifications.
Proteins are the real-time executors of many biological functions and proteomics is the large-scale study of proteins, including their structures, localizations, post-translational modifications (PTMs), and functions.
Proteomics experiments also provide information on protein interactions and complex formation. For example, proteins interact with each other as part of large complexes that serve to execute most biological processes including signal transduction, transcription, and translation. A literature search at the start of 2013 showed there were 38031 articles published on proteomics encompassing several research strategies; today, after four years, their number is more than doubled to a value of more than 86000 articles. In both bacteria and eukaryotes, the cellular concentrations of proteins do not completely correlate with the abundances of their corresponding mRNAs. They often show a squared Pearson correlation coefficient of about 0.40, this means that about 40 % of the variation in protein concentration can be explained by knowing mRNA abundances. This

demonstrates that proteomics represents a complementary to genomics approaches.

The classic proteomics screening methodology combine two different approaches. The first one, called expression-based proteomics, has the aim to define the expression of all proteins present in biological samples. Traditionally, it is performed through the combination of several sequential steps including protein extraction, separation and identification. The general starting point is the protein separation by an electrophoresis system, one- or two-dimensional electrophoresis (1-DE or 2-DE), and the subsequent identification of digested proteins by MS. Alternatively, proteins can also be digested using a specific protease and the resulting peptides separated and analysed immediately by MS. Such approach, namely as shotgun, is considered the method of choice for the large-scale analysis of proteins. The strength of this approach is that it is unbiased; a drawback is that the outcome relies on analysis and interpretation of experimental data. By contrast, targeted proteomic using multiple-reaction monitoring mass spectrometry (MRM-MS) allows the selective detection and quantification of selected peptide ions. Such approach uses the capability of triple quadrupole mass spectrometers to act as ion filters. In a MRM-MS experiment, the precursor ion is isolated in the first quadrupole (Q1), fragmented within Q2 producing fragment ions that are monitored using Q3.

The second approach, functional proteomics, aims to define the biological role of proteins and to identify protein–protein interactions, or interactomes. Protein complexes can be purified in several ways, one very common approach is to use an affinity tag to the protein of interest and purify the interacting partners.

Proteomics has emerged more than two decades ago as a post-genomic technology with the promise to unravel the cellular mechanisms of diseases and to develop reliable markers of diagnosis or treatment. However, such studies remain challenging owing to the high degree of complexity of cellular proteomes, in particular the serum/plasma proteome, and the low abundance of regulatory proteins hidden by abundant proteins. Due to the enormous variation in protein diversity, there is currently no single methodological platform that can be used for a full characterization of the proteome.

2.1. Methods for protein separation

The separation of all the proteins contained within cells, tissues, and biofluids remains a challenging analytical problem. Existing methodologies are not adequate to completely isolate and resolve the large number of proteins present at such different levels of concentration. Proteomic approaches can be classified as either gel-based or gel-free methods that can be further subdivided in "label-free" or "label-based".

2.1.1. Gel-based Proteomics: 2-DE

The attempts to develop a 2-DE started in the late 60', but it was O'Farrell in 1975 who optimized a method on the basis that each electrophoresis separation must be done in independent parameter, to avoid protein being distributed across a diagonal rather than across the entire surface of the gel (Magdeldin 2012). In the first dimension, protein molecules are resolved depending on their isoelectric point (pI); in the second dimension, protein separation is performed based on molecular weight (Magdeldin et al. 2014).

This technique has broadly affected life science research and successfully used applied to the study of biological or clinical samples for the purposes of identifying novel disease-specific protein biomarkers or gaining better understandings novel protein targets for therapeutic interventions and drug developments. During these years, several advances that have enhanced resolution, detection, quantitation, and reproducibility of the technique, increasing the robustness of the entire 2-DE workflow. One of the most notable improvements was the introduction of immobilized pH gradient (IPG) gels that led to standardized procedures for 2-DE permitting higher resolution and improved reproducibility for inter laboratory comparisons. More recently, the development of 2-D differential in-gel electrophoresis (DIGE) in 1997 overcame problems of reproducibility and quantitation because allowed running test and control sample in the same gel. This method was designed in an attempt to increase sensitivity and reproducibility of 2-DE using multiplexed fluorescent dyes- labelled protein samples. 2D-DIGE is based mainly on running more than one sample (maximum 3) on a single

gel at once. Different fluorescent cyanine (Cy) dyes are used for labelling proteins from different samples

This technique enables protein detection at sub picomolar levels and relies on pre electrophoretic labelling of samples with one of three spectrally resolvable fluorescent CyDyes (Cy2, Cy3, and Cy5) (Abdallah et al. 2012). Images are subsequently imported into dedicated 2-DE image analysis softwares.

Although there has been a significant progress towards liquid chromatography and MS methods to separate and analyse proteins, 2-DE still remain a popular technique for conducting proteomic studies. Proteins of interest are excised from the gel, proteolytically digested, and identified using MS (Figure 1). In a single run, up to 1,000 – 2,000 protein species from one complex sample can be separated. In specialised laboratories, using large-gel 2-DE method, the number of protein spots detected were drastically increased up to 10, 000. Subsequently, 2-DE gel easily and efficiently couples with many other analysis and biochemical techniques; spots can be excised, proteins can be extracted and after a tryptic digestion analysed by mass spectrometry.

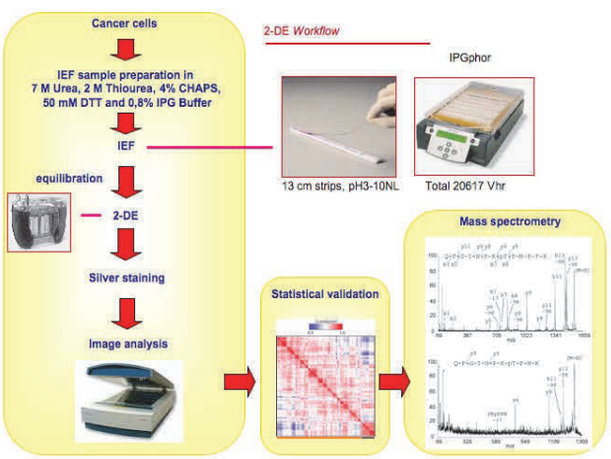

Figure 1. A schematic workflow of two-dimensional gel electrophoresis (2-DE). 2-D gel electrophoresis is an experimental technique that combines two separation methods. Biological samples are grown under different conditions and total proteins are extracted and subjected to isoelectric focusing (IEF) (first-dimension electrophoresis), where proteins are separated according to their isoelectric point (pI). After first dimension, IPG-strips are re-equilibrated to the second dimension buffer conditions, and transferred to the SDS-PAGE gels. Proteins on gels are visualized by MS-compatible stains, including Coomassie or silver staining. Software-based image analysis is then crucial for the biological interpretation of experiments. After statistical validation, differentially expressed spots from 2-DE gels are excised, and a tryptic digestion is performed to generate tryptic peptide mixtures of the proteins that are applied to MALDI- or LC-MS/MS for identification of the excised proteins. The peptide data then are compared with the entire protein database (Swiss-Prot, NCBI).

2.1.2 Gel free-based approaches

Because of problems in quantitative reproducibility and limitations on the ability to study certain classes of proteins, researchers have developed alternatives to 2-DE, for MS-based proteomics techniques.

High-resolution liquid chromatography (LC) separation coupled on line with a mass spectrometer is the central component of a gel free-approach. Complex protein mixtures are digested by trypsin into polypeptides, which are then separated by LC and analyzed by MS via an electro spray ionization (ESI) interface.

In this approach is not the protein itself, which is separated and identified. Instead, proteins are cleaved into peptides using proteolytic enzymes and, subsequently, these peptides are separated and subjected to mass spectrometric analysis. This allows the determination of the protein content of the initial sample (Baggerman et al. 2005).

For this purpose, chromatographic separations are performed using flow rates in the range of low nanoliter per minute (nano-flow liquid chromatography or nanoLC). The relative quantification of peptides usually involves either label-free or stable isotope labelling techniques to identify differences in protein abundances. The labelling methods can be classified into two main groups: chemical isotope tags and metabolic labelling. A variety of labelling approaches including, Proteolytic Labelling, Isotope-Coded Protein Labelling (ICPL), Isotope-Coded Affinity Tags (ICATs), Isobaric Tags for Relative and Absolute Quantification (iTRAQ), TandemMass Tag (TMT), 14N/15N Labelling and Stable Isotope Labelling by Amino Acids in Cell Culture (SILAC) are valuable techniques in quantitative proteomic analysis. The rationale behind each labelling strategy is to create a mass shift that distinguishes identical peptides that exhibit the same chromatographic and ionisation properties, from different samples within a single MS analysis.

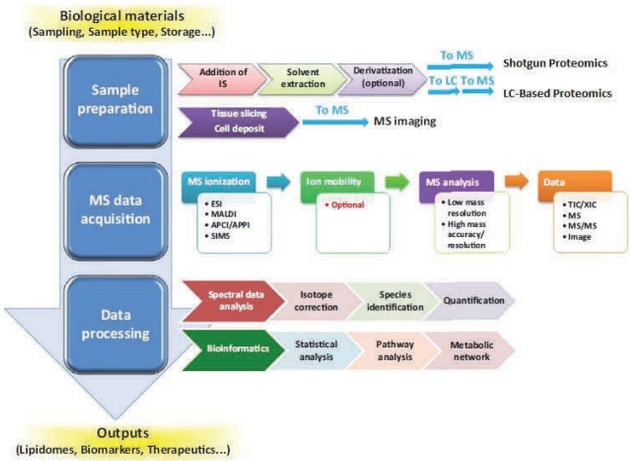

Figure 2. A schematic workflow of gel free electrophoresis (Yang and Han 2016)

3. Limitations of current proteomics approach

In recent decades gel based proteomics techniques became robust and reproducible, however two distinctive issues remains a challenge: the problems to detect low abundance and hydrophobic proteins.

The question of under-representation of hydrophobic protein and in particular membrane proteins is well known and to explain this problem very different possible mechanisms have been proposed: (i) aggregation caused by the low solubility of these protein species in the aqueous media; (ii) protein loss over the sequential steps of the 2-DE processes; (iii) precipitation at the protein corresponding pI during isoelectrofocusing phase; (iv) expression in low copy numbers; (v) difficulties to identify them by MS than hydrophilic proteins. Several fruitful strategies were considered to solve the problem. Usually, the best strategy in 2-DE experiments is to solubilize proteins from the lipid layers by detergent and chaotropic salt. This can be performed by applying a solution of 2M thiourea, 8M urea, and 4% chaps. In the most recent years, other solutions were proposed: use of different zwitterionic detergents, nonionic n-dodecyl β-D-maltoside and zwitterionic amidosulfobetaine ASB-14, and 1,2-diheptanoyl-sn-glycero-3-phosphatdiyl choline (DHPC). Two organic solvents have been recommended for miscible extraction of red blood cells membrane proteins using methanol (MeOH), 2,2,2-tri- fluoroethanol (TFE) and urea.

Despite the many solutions proposed in the recent years the problems with hydrophobic proteins, on 2D gels are widely unsolved. In fact, membrane protein solubility is low at their pI, and therefore membrane proteins tend to precipitate at their pI range. It is evident that the issue represents a built-in problem for all 2D electrophoresis systems IEF-based, for this reason IEF-free separation systems represent a natural alternative in the analysis of membrane proteins.

3.1 Protein abundance

The large number of gene and gene splice-variants that encode proteins, as well as the extensive post-translational modifications of eukaryotic proteins renders proteomic studies extremely difficult. The detection of specific, disease-related protein markers, notoriously difficult to identify, because expressed at low concentration, can be extremely challenging on a classical proteomic experiments where highly abundant proteins could obscure the rare ones. Consequently, there has been an extensive investment into developing techniques and methods capable of revealing the so named "hidden proteome". This cannot be achieved by one single approach. In fact, several methods are used for the enrichment and visualization of the low-abundance proteins and also for the depletion of the high-abundance proteins.

It was demonstrated that the 10% most-expressed gene products represented the 75% of the total protein content, and the 2/3 of less-expressed only 10% of the protein content. In this situation is simple to argue that the signal of high-abundance proteins tends to hide the signal of rare species.

Some of these technical difficulties can be bypassed loading more sample, exploiting the great capacity of 2D gels, allowing many of low abundant proteins to be detected because above the detection limit. But high-loading approach is restricted by gel crowding and is related to the strong presence of normal and modified forms of high abundance protein species. This strategy gives gel with completely saturated zones with no increased performance in the visualization of low abundant species. Possible solutions proposed the use of giant gels with greater resolution and capacity. However, this

technology is inadequate and difficult to use due to the extreme fragility of the gels employed in the analysis.

To address these issues, analytical chemists have attempted to develop pre-fractionation methods to separate large numbers of proteins. Fractionation based-methods that take advantage of proteins function or structure are extensively used, allowing the isolation of specific proteomes: glyco-proteome by lectin columns, phospho-proteome by anti-phospho-aminoacid antibodies or metal-chelating resins. However, these protocols do not resolve the challenge of signal suppression due to high-abundance species present. Immunodepletion columns, containing immobilized antibodies addressing the highest abundance proteins, were proposed as a possible solution. Though, these approaches cause the dilution of the initial sample, rendering it even more difficult to detect low-abundance proteins.

In this scenario, combinatorial hexapeptide ligand libraries have arisen as a powerful method for sample handling and are recently used to better elucidate and obtain extensive information on the protein composition of complex samples like serum, bile fluid, human urine, platelet extracts, and red blood cell lysate. The ligand libraries, designed as batch of chromatographic beads, are synthesized by modified Merrifield approach described by Lam and collaborators. The library consists of millions of affinity baits (hexapeptides) so that each bead comprises multiple copies of the same bait. The beads represent the affinity solid phase of chromatographic column. This hexapeptide ligand library is assembled in order to be able to bind each single protein species present in a given biological extract. On the basis of the saturation-overloading chromatographic principle the loaded proteins are captured by their respective specific ligand until saturation whereas the excess, unbound proteins are washed away. The proteins are captured from the peptide library by several and different combination of interacting forced: Van der Walls interactions, hydrogen bonding, structural docking, hydrophobic associations etc. Thus, high represented proteins species rapidly saturate their specific bead ligands while the excess of the same protein remains unbound. On the contrary, low-abundance proteins were concentrated by their ligand up to saturation. Washing steps eliminate the protein excess not bound to the library and are removed from the chromatographic column. The protein species bound are eluted by a single appropriate elution buffer able of destroy proteins–hexapeptides interaction or by a sequence of desorbing agents, each of them addressing a selected type of binding. These fractions can then be analyzed using well-known methods, such as SDS-PAGE, 2D electrophoresis and MS. An application of hexapeptide libraries to cellular lysates is reported in Figure 2. Proteins obtained from the human T cell lymphoblast-like cell line Jurkat were used as starting material to show the feasibility of this approach.

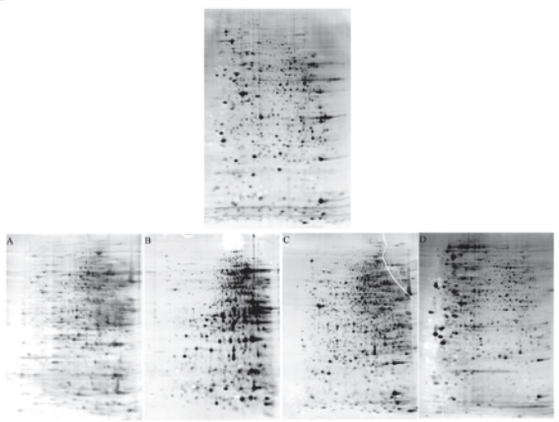

Figure 3. Figure shows the 2-DE maps (pH 3-10) obtained from initial Jurkat cell line extract (top) as compared with those obtained from: (A) 2.5 mg/ml of proteins treated with library and eluted with RS solution (2 M thiourea, 7 M urea, 4% CHAPS); (B) 5 mg/ml of proteins treated with library and eluted with RS solution (2 M thiourea, 7 M urea, 4% CHAPS); (C) and (D) 14 mg/ml of proteins treated with library and eluted with RS solution (2 M thiourea, 7 M urea, 4% CHAPS) and RSA solution (2 M thiourea, 7 M urea, 4% CHAPS, acetic acid to pH 3.3), respectively.

Jurkat cell line was maintained in RPMI-1640 medium containing 10% fetal bovine serum (FBS) and antibiotics. Cells were harvested by centrifugation followed by lysis via sonification in Tris buffer containing protease inhibitors. Varying amounts of cell lysate (2.5 mg/ml, 5 mg/ml, 14 mg/ml) were subjected to column chromatography over a solid-phase combinatorial ligand library (ProteoMiner, Biorad). Following washing, each individual column was subjected to two distinct elutions using a RS solution (2 M thiourea, 7 M urea, 4% CHAPS) and a RSA solution (2 M thiourea, 7 M urea, 4% CHAPS, acetic acid to pH 3.3) respectively. RSA eluted sample was precipitated with 2D Clean-Up (GE Healthcare) and resuspended in RS.

For 2-DE studies, 80 µg of proteins were dissolved in sample buffer and isoelectric focusing of protein samples was carried out by using commercial 13 cm IPG polyacrylamide strips (pH 3 to 10 NL). Separation in the second dimension was carried out in 12% SDS-PAGE gels. Silver stained gels were scanned and analyzed by the software Image-Master 2-D Platinum.

4. Bioinformatics for high-throughput platforms

Over the last decades, large-scale genome sequencing of model organisms and humans (Lander et al. 2001) has been a landmark achievement in biomedical sciences that was fueled by extraordinary advances in molecular biology and computer science. The broad application of genomics and proteomics fields as well as the diffusion of high-throughput platforms, have led to increasing the volume of available data requiring efficient algorithms along with new data management, new analysis and novel visualization techniques. This is in turn helped in redefining the synergy between biology, information sciences, mathematics, and computational techniques. This synergy is largely used by researchers to analyze the information on a large-scale, through the integration of computational algorithms, software tools and databases in an effort to address biological and medical questions.

Luscombe et al. proposed a definition on bioinformatics as a union of biology and informatics: it involves the technology that uses computers for storage, retrieval, manipulation and distribution of information related to biological macromolecules such as DNA, RNA, and proteins (Luscombe, Greenbaum, and Gerstein 2001).

Initial research applications of bioinformatics were primarily focused on analysis of biological sequence data, genome content and rearrangement, as well as for prediction of function and structure of macromolecules (Luscombe, Greenbaum, and Gerstein 2001). Over the last few decades, with the advent of high throughput "omics", it is now feasible to systematically profile a biological system at different levels of molecular and cellular organization, including its epigenome, transcriptome, metabolome, proteome, and interactome (Joyce and Palsson 2006). Overtime, then, the broad nature and scale of these data types have spawned from conventional bioinformatics to specialized branch of bioinformatics such as comparative genomics (Miller et al. 2004), functional genomics (Vukmirovic and Tilghman 2000), network biology (Barabási and Oltvai 2004) and computational proteomics (Colinge and Bennett 2007). Moreover, with the subsequent realization that the complete understanding of biology is obtained from multi level data integration, computational system biology has come into prominence.

Mass spectrometry based proteomics has undergone important advances in the scientific community, due to technological instrumentation and innovation in computational proteomics (Cox and Mann 2007) that is am intensive discipline that requires extensive analytical and data-mining support and bioinformatics approaches.

We can distinguish between qualitative proteomics and quantitative proteomics. In qualitative proteomics, most of the bioinformatics activities are focused on functional data mining of the dataset to extract the global biological theme underlying the proteome. The first step for functional interpretation of a protein list is to connect the protein identifier with its associated Gene Ontology Terms (GO_terms). Genes are then associated to hierarchically clustered, functional terms that describe the "biological process", "molecular function" or "cellular component" (Schmidt 2014). Similarly to GO term enrichment analysis, proteins can also be analyzed for pathway abundance which might be more meaningful since allow the identification of functional biological processes associated to that protein (Schmidt 2014). Comprehensive pathway databases such as KEGG, include many interaction data coming from intracellular reactions such as metabolism or signaling pathways, genetic alterations or drug studies (Kanehisa et al. 2012).

Genome-wide annotational datasets like GO, protein domain organization (PFAM, InterPro) (Mulder et al. 2007), pathways (KEGG) (Kanehisa et al. 2004) and disease mutations (OMIM) have been successfully used for functional proteomics (Pasini et al. 2006). These annotations can be further used in conjuction with statistical tests to find over/under-represented functional categories (Adachi et al. 2007). Additionally, integration with other high throughput "omics" datasets, such as microarray, may provide valuable insights into proteome expression and disease mechanisms (Graumann et al. 2008).

In quantitative proteomics, we know that mass spectrometry provide quantitative data of proteome changes in the cellular states. This information can be used on a binary level of protein changes, for instance normal vs cancer,

stimulated vs non-stimulated cells with growth factors as well as a step of cell cycle or differentiation. There are a lot of methods and approaches for proteomics datasets across multiple conditions or samples. However, in clinical applications of proteomics, biomarker discovery can be done by comparison of proteome profiles of healthy and disease profiles, using genetic algorithms (Petricoin et al. 2002) and on building classification models for disease predictions.

Microarrays have now permeated literally every field of biology and have found many applications in translational research. Large-scale microarray data are also becoming crucial for experimental biology along with computational algorithms, which find wider application in basic research, target discovery, drug discovery, biomarker identification and disease determination. Microarray Bioinformatics include, indeed, a range of inferential (Rhodes et al. 2002) and descriptive statistical methods. Reverse engineering of gene networks using gene expression data based in Bayesian statistics (probabilistic models) (Friedman et al. 2000), Boolean and Relevance networks (Butte and Kohane 2000) and graph theoretic algorithms (Tringe, Wagner, and Ruby 2004) are successfully applied to microarray data to uncover patterns in disease, especially in cancer (Lapointe et al. 2004).

Bioinformatics approach for identification of cis-regulatory sequences has gained impetus in computational prediction of the gene regulation, especially from recent discoveries in transcriptional regulation by regulatory RNA (miRNA, siRNA, piRNA) and their targets (Bentwich 2008) as well as the current appreciation of the role of epigenetics. Bioinformatics applied to gene regulation is one of the most exciting areas of computational research. One of the principle means of coordinating transcription spatially and temporally is through the presence of cis-regulatory elements. These short DNA sequence motifs, proximal to the transcriptional start site (TSS), are bound by transcription factors responsible for the recruitment of the transcriptional initiation machinery and represent principle components that act in response to a particular cellular context and extra-cellular inputs. Global elucidation of gene-specific cis-regulatory control will permit the understanding of global transcriptional networks and facilitate our understanding of the properly functioning of biological system. In addition, de novo prediction of these binding sites has become an active area of research in the field of functional genomics. A complete understanding of this molecular algorithm give great impact on biological research, essential for gaining insights into development, cellular responses to environmental and genetic alterations and the molecular basis of many diseases.

The dynamic interplay between genes, proteins and metabolites leads to a complex biological network that include protein-protein interactions, regulatory circuits linking transcription factors, cis-regulatory elements, signal transduction pathways and metabolic pathways. Network bioinformatics is a fascinating areas of bioinformatics and aims to study this complex biological circuit using mathematical modeling and simulation of pathways, graph-theory analysis of global network structure, application of engineering concept of network analysis as well as de novo design of networks. Protein-Protein interaction network, are typically visualized as "graph networks" where the nodes represent the protein, while an edge connecting two nodes. Protein-Protein interactions are often displayed as a networks illustrating the high degree of connectivity and the presence of particular hub proteins.

Genomics and bioinformatics are now poised to revolutionize our healthcare system by developing personalized and customized medicine. The high speed genomic sequencing coupled with sophisticated informatics technology will allow clinician to quickly sequence a patient's genome and easily detect potential harmful mutations and to engage in early diagnosis and effective treatment of diseases (Xiong 2006).

5. Clinical applications of proteomics

With the advent of proteomics several large-scale studies were launched to investigate the protein profile in different biological systems with the aim of discovering potential diagnostic and prognostic biomarkers. For example, in 2000, the National Cancer Institute (NCI) established an initiative titled the Early Detection Research Network (EDRN) which the objec-

tive to facilitate the development of biomarkers or technology that enable early detection of cancer.

Specifically, this technology offers the possibility of identifying and quantifying proteins associated with a particular disease by means of their altered levels of expression and/or PTMs between the control and disease states. This type of comparative analysis enables correlations to be drawn between the range of proteins, their variations and modifications produced by a cell, tissue and bio-fluids and the initiation, progression, therapeutic monitoring or remission of a disease state. Then, clinical proteomics should be defined as the application of proteomic analysis with the aim of solving a specific clinical problem within the context of a clinical study. The potential applications of proteomics goes from metabolic syndromes like inflammatory diseases or diabetes, to neurological disorder, cancer, dietary interventions, to drug discovery or screening (Yang and Han 2016).

As clinical proteomics consists of a variety of experimental procedures, pre-analytical variability, as well as analytical and post-analytical procedures can markedly affect a proteomic experiment. Collection of appropriate clinical specimens (e.g. urine, blood, tissue), duration of storage, number of freeze-thaw cycles, analysis of proteins and peptides of interest, data interpretation, data validation of protein dataset in a specific clinical context should effectively be standardized to reduce bias. As a result, recommendations concerning minimal information about a proteomic experiment (MIAPE) were released from the Human Proteome Organisation (HUPO). Reference materials are also expected to support both qualitative and quantitative proteomic measurements.

Despite substantial progress in the field, clinical proteomic approaches have not matured into routine diagnostic applications. As described above, proteomic analysis of blood and other body fluids and tissues is extremely difficult due to the complexity of samples and the dynamic range of concentrations of proteins in biological fluids. Major challenges exist for plasma biomarkers discovery, where the large dynamic concentration range of up to ten orders of magnitude for plasma proteins and the presence of very high abundance proteins such as serum albumin and immunoglobulins mask the lower abundance plasma biomarkers. Moreover, to validate biomarkers in a clinical setting it is required the analysis of hundreds (and perhaps more) of high-quality clinical samples. In fact, a huge amount of data and samples is necessary to ensure a bio-statistical significance. This large set of samples is in contrast with the time consuming and intensive proteomic approach that are distant from the routine of a clinical laboratory.

Improvements in this field could lead the way to the use of "omic sciences" as a diagnostic tool for screening and early detection of many pathologies. But they also could represent prognostic and predictive tools, providing information about the outcome of diseases like breast cancers; triple negative tumours have a very worst prognosis than hormone responsive ones (Crutchfield et al. 2016).

One of the major factors for successful proteomic analysis of clinical samples is the selection of an appropriate workflow. For instance, in studies that use biological fluids, samples should be pre-treated to remove high-abundance proteins (running the risk also to eliminate proteins of interest because of protein-protein interactions) or concentrated to enrich the protein fraction (in the case of urine). In biomarkers discovery, caution must be exercised in preserving samples for protein degradation, a problem that can lead to misinterpretation of data.

Working with proximal fluids (synovial fluid, pleural fluid, peritoneal fluid, ascites) it should be necessary to eliminate the contamination of mucosa and salts before sample separation by 2DE-MS, LC-MS), or capillary electrophoresis coupled to mass spectrometry (CE-MS).

With regard to clinical proteomics, among the strategies that have the highest potential to reduce the gap between proteomics and its clinical application there is the possibility to conduct a differential proteome analysis on tissue samples with the advantage to investigate the disease directly at the origin. Moreover, biomarkers present in tissue are more concentrated than those released in the blood making this biological sample suitable for specific isolation or fractionation schemas. However, researchers who work with this type of sample are well-aware that the great heterogeneity of human tis-

sues represents a well-known limit in the investigation of biomarkers. Several approaches were developed to overcome this problem with the potential to be clinically useful. 2D electrophoresis or SELDI (surface-enhanced laser desorption/ionization) have been coupled to laser capture microdissection (LCM), allowing the precise procurement of enriched cell populations from a heterogeneous tissue, or live cell culture, under direct microscopic visualization, or laser microdissection and pressure catapulting techniques (LMPC). In this last procedure, after microdissection, the sample is directly catapulted into an appropriate collection device. As the entire process works without any mechanical contact, it enables pure sample retrieval from morphologically defined origin without cross contamination.

Among the strategies that have the highest potential to reduce the gap between proteomics and its clinical application there is the possibility to conduct a differential proteome analysis directly on tissue samples with the advantage to investigate the disease directly at the origin. In these years, MALDI Imaging has emerged has another promising technique for the combined morphologic and molecular tissue analyses. In detail, MALDI Imaging allows to image/profile intact tissue sections placed onto a conductive glass side obtaining information about protein expression and localization. Because of its practical simplicity and ability to obtain reliable information from tissue section, MALDI imaging might have the potential to complement histopathologic evaluation for assisting in diagnostics, patient stratification, or predicting drug response.

Together with technological challenges, other issues that could affect proteomic results should not be underestimated including harvesting, handling and storage of samples. Considering that the proteome is dynamic over time and expression of a myriad of factors, researchers should also consider the clinical history of the patient such as age, sex, and race. To minimize these systemic problems is desirable to establish a specimen bank (biorepository). A sample, to become eligible for a biorepository, must be collected and analysed immediately because cells and proteins degradation and /or modification may affect the analysis. Moreover, sample should be subject to an accurate quality control and catalogued according to trusted, safe and standardized clinical data.

Based on these observations we conclude that proteomics can be considered as a main strategy for biomarker discovery. However, special attention has to be paid to reduce pre-analytical variables, analytical variability, and biological variation. This will require a close interdisciplinary collaboration involving clinicians, statisticians / bioinformatics, epidemiologists, chemists, biochemists and biologists.

6. Omics science: toward omic personalized medicine

The availability of human genome sequence has transformed biomedical research over the past decade. The end of the 20th century was marked by the genomics revolution. However, over the past decades it has become clear that common diseases develop as a result of multiple defects at different levels including proteins, lipids and metabolites, defects can that cannot be completely predicted by the simple analysis of genes. A systems-level approach, that integrate the results of genomics with those obtained by the analysis of metabolomes and proteomes, has enabled researchers to utilize novel strategies to tackle unexplored research questions in human diseases. The ultimate goal is to evolve an integrated omics picture of the genes, transcripts, proteins, and metabolites to fully describe cellular functioning. At this regard in 2014, a draft map of the human proteome was realised, using the high-resolution Fourier-transform mass spectrometry (Kim MS et al. Nature 2014). In that review was reported a proteomic profiling of 30 histologically normal human samples, including 17 adult tissues, 7 fetal tissues and 6 purified primary haematopoietic cells, that resulted in the identification of proteins encoded by 17,294 genes accounting for approximately 84% of the total annotated protein-coding genes in humans. The proteogenomic analysis revealed a number of novel protein-coding regions, which includes translated pseudogenes, non-coding RNAs and upstream open reading frames. This large human proteome catalogue (available as an interactive web-based resource at http://www.humanproteomemap.org) will complement available human genome and

transcriptome data to accelerate biomedical research and in particular in the diagnosing and treating of human diseases

The greatest benefits for patients are likely to be realized from the monitoring and management of early stage disease rather than from treatment of late stage disease. In this field, a comprehensive integrative omic profiles was applied with success to perform an integrated Personal Omics Profiling (iPOP) on a single healthy individual. Authors of this study combined genomic, transcriptomic, proteomic, metabolomic, and autoantibody profiles, in conjunction with routine laboratory testing, from a single individual over a 14 months period, generating an individual iPOP over the course of healthy states and two viral infections that occurred during the study interval. Peripheral blood mononuclear cells, plasma and serum were collected and results from whole-genome sequencing predicted an increased disease risks for various diseases, including hypertriglyceridemia, and type 2 diabetes (T2D). Markers associated with T2D became elevated during the course of study, in particular, following a respiratory infection. Moreover, data from omics experiments before and after viral infections allowed for the creation of a dynamic picture of this process.

Omics-based approaches will identify at risk groups supporting the implementation of risk-stratified health screening. This may lead to significant cost-savings at the societal level.

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Summary box

Given the complexity of cellular systems, several techniques have been developed over the years for the comprehensive analysis of molecular components. In particular, the advent of omic sciences has changed the way in which human diseases are studied making possible the simultaneous interrogation of thousands of molecular species at the system level. It was a technological revolution that modified the way in which experiments are designed, moving from mostly hypothesis- based approaches to studies that are largely hypothesis free. In this book chapter, we briefly discuss classical omics approaches including next-generation sequencing and metabolomics. More in detail, we have opted to focus our attention on proteomics, a complementary approach to genomics that over these years has led to important insights in the comprehension of cellular biological processes and human diseases. Current experimental limitations including the enormous complexity and the dynamic nature of proteomes are also discussed.

When integrated among them, omics approaches have the great potential to provide insight into the molecular alterations that drive disease pathogenesis.

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Mitochondria and fertility: the mitochondria critical role on spermatozoa function

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Abstract

Mitochondria of spermatozoa significantly differ, structurally and functionally, from the corresponding organelles of somatic cells. Nevertheless, sperm mitochondria, as well as the somatic ones, are the location of the oxidative phosphorylation (OXPHOS) process, which is necessary for the production of metabolic energy in the form of adenosine triphosphate (ATP). In addition to their basic role in oxidative energy generation, mitochondria are also a source of reactive oxygen species (ROS), which, at low concentrations, play a physiological role in many sperm processes.

It is commonly accepted that a proper functionality of mitochondria is necessary for a high quality of sperm cells; this last parameter, in its turn, is a prerequisite for a high sperm fertilizing ability.

Evaluation of mitochondrial respiratory efficiency represents therefore a valuable test that could integrate routine semen analysis.

Keywords: mitochondria, spermatozoa, oxidative phosphorylation, ROS, sperm motility

1. Introduction

Mitochondria are cellular cytoplasmic organelles which take part in a variety of cellular metabolic functions. They are involved in several pathways, such as the Krebs cycle, the oxidative decarboxylation of α-ketoacids, the β-oxidation of fatty acids, many reactions of the amino-acid metabolism and of the pyrimidine synthesis. Furthermore, mitochondria are actively implicated in other processes, such as cell differentiation, ROS generation, apoptosis, calcium signalling, iron metabolism, etc. However, these organelles are generally known as the energy-generating powerhouses of the cell, because they play a fundamental role in the production of adenosine triphosphate (ATP) through the sophisticated mechanism of the oxidative phosphorylation (OXPHOS).

This last process, which is the topic of this manuscript, requires the coordinated operation of two main components: the respiratory chain and the ATP-synthase, both located in the inner mitochondrial membrane.

The mitochondrial respiratory chain is involved in the transport of reducing equivalents from some electron-donors to the molecule of oxygen with the final formation of water. The energy released from these oxidation/reduction reactions is used to drive the synthesis of ATP from ADP. A strict coupling is therefore required between respiration, which is the electron transfer through respiratory chain complexes, and phosphorylation, which is necessary to synthesize ATP.

In addition to their basic role in ATP synthesis, mitochondria are a major source of reactive oxygen species (ROS), which are key mediators of cellular physiology and pathology.

Mitochondria of spermatozoa are different from the corresponding organelles of somatic cells, in both their morphology and biochemistry (Piomboni et al., 2012; Ferramosca and Zara, 2015).

They are helically arranged around the midpiece of sperm and show a peculiar morphology and arrangement. The biochemical difference is essentially related to the existence of specific enzyme isoforms, which are characterized by peculiar kinetic and regulatory properties.

It has been demonstrated that a proper functionality of mitochondria is necessary for a high quality of sperm cells and, in particular, for sperm motility. According to this hypothesis, structural and functional alterations are usually found in mitochondria from asthenozoospermic subjects.

However, not only motility but also several essential sperm functions require ATP as an energy source. Therefore, a careful and detailed investigation of mitochondrial bioenergetics of spermatozoa could provide more insight on the role of these organelles in the overall quality of the gametes. We are confident that evaluation of mitochondrial respiratory efficiency could integrate routine semen analysis in clinical investigation of male infertility.

2. A reliable tool for the evaluation of sperm respiratory efficiency

In 2008 we reported a relatively simple and fast method for analysing oxygen consumption, and therefore mitochondrial functionality, in individual human ejaculates (Ferramosca et al. 2008).

Human spermatozoa were incubated in hypotonic buffer to selectively disrupt the plasma membrane and were subsequently used for studies of mitochondrial respiration. The rupture of the plasma membrane, followed by the washing steps, caused the loss of the various metabolites contained inside the cells, thus exposing sperm mitochondria to an environment with a well- defined composition. At the same time, mitochondria maintained their intactness and functionality.

Oxygen uptake by hypotonically-treated spermatozoa was therefore measured at 36°C by using a Clark-type oxygen probe, in the presence of respiratory substrates (10 mM pyruvate and 10 mM malate) and 0.76 μM adenosine diphosphate (ADP) (Fig. 1). The ratio between the rate of oxygen uptake in presence of respiratory substrates plus ADP (V_3) and the rate of oxygen uptake in presence of the substrates alone (V_4), allowed for the calculation of a respiratory control ratio (or RCR).

In normozoospermic samples RCR was about 2.5, hence indicating a good coupling between respiration and phosphorylation. Interestingly, whereas the rates of oxygen uptake, as expected, changed with different sperm concentrations, the RCR values remained constant, thus demonstrating a linear response of the assay. The limit of sensitivity of this experimental system was found to be 1.5 x 10^7 sperm cells.

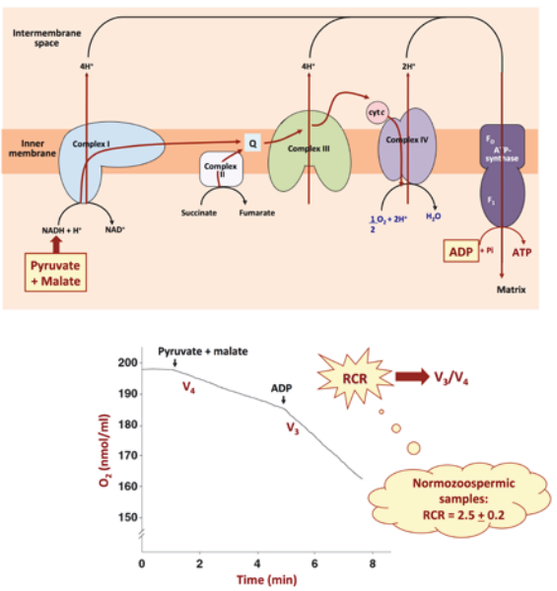

Figure 1: Assay of oxygen consumption in sperm cells

In order to test the possibility of a difference in mitochondrial function between progressively motile and less active sperm, we measured the respiration capacity of human sperm mitochondria from some asthenozoospermic subjects with a mean percentage of sperm motility of 36%. In these samples we found a significant reduction in the active state of respiration (V_3) when measured in the same experimental conditions used for normozoospermic samples. On the contrary, the V_4 value was almost unaffected, thus leading to a significantly lower value of RCR in asthenozoospermic subjects in comparison with the normozoospermic ones (Ferramosca et al. 2008).

The importance of mitochondrial functionality was also extended to hyperactivated motility observed during sperm capacitation. We meas-

ured the respiration capacity of human sperm mitochondria before and after swim up treatment (Stendardi et al., 2011). In sperm samples selected by swim up, we found a significant increase of about 10 fold in the V_3 and V_4 values. These results suggest that sperm motility strongly depends on mitochondrial respiratory function. Furthermore, high values of V_3 and V_4 obtained with sperm samples selected by swim up suggest that our experimental system responds also with a limited amount (less than 5 million) of sperm cells.

3. Sperm motility and mitochondrial respiratory efficiency

In a following study, we correlated sperm mitochondrial respiration, evaluated by the polarographic assay of oxygen consumption and RCR values, with variations in sperm motility (Ferramosca et al., 2012). Interestingly, we found a profile for RCR values with respect to a gradual increase or decrease in sperm motility (Fig. 2)

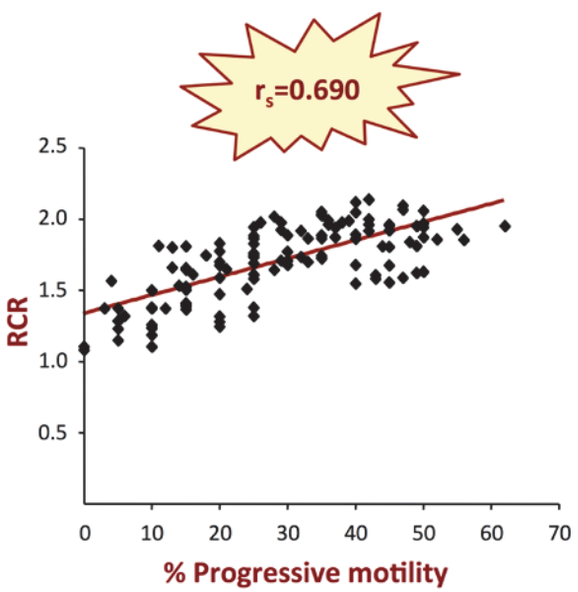

Figure 2: Sperm motility vs RCR values

In particular, V_3 (active state of mitochondrial respiration) positively correlated with progressive sperm motility, whereas V_4 (resting state of mitochondrial respiration) showed a lower positive correlation with sperm motility.
In the same study we also investigated a possible relationship between RCR values and the percentage of morphologic anomalies affecting the head, midpiece, or tail of spermatozoa. Interestingly, a strong negative correlation between mitochondrial respiration and defects in sperm midpiece, where mitochondria are exclusively localized, was found, suggesting that structural anomalies were accompanied by a parallel decrease in mitochondrial respiration. In agreement with these results, the anomalies in the sperm midpiece were also associated with decreased sperm motility.

4. Oxidative stress and sperm mitochondrial respiratory efficiency

For proper functionality of spermatozoa, an adequate level of ROS is required. Free radicals are involved in sperm hyperactivation and capacitation, acrosome reaction, spermatozoa-oocyte fusion, and other molecular events implicated in human fertility. However, high levels of ROS have negative influence on sperm quality and function.
What is the relationship between ROS and sperm mitochondria?
In this context we analyzed the dependence of mitochondrial respiration efficiency on seminal lipoperoxides (LPO) levels. LPO are a specific indicator of oxidative stress in seminal fluid. We found a strong negative correlation of RCR values with seminal LPO, suggesting that an increase of oxidative stress in seminal fluid (which is associated to an increase in the levels of LPO) was able to impair mitochondrial functionality.
Sperm DNA damage could be a marker of sperm quality and is often associated to oxidative stress. The analysis of dependence of sperm mitochondrial respiratory efficiency on sperm DNA fragmentation showed a strong negative correlation between RCR values and the percentage of DNA fragmentation.
When we analyzed V_3 and V_4 values, we found a moderate dependence of V_3 (which is the rate of oxygen consumption after the addition of ADP) on the levels of LPO and a strong correlation between V_4 and LPO. V_4 shows also a significant dependence on sperm DNA fragmentation, while V_3 seems to be independent.
We can therefore hypothesize that a condition of oxidative stress in the seminal fluid (which is

associated to an increase in the levels of LPO and in the percentage of DNA fragmentation) produces an increase in V_4, suggesting a stimulus of mitochondrial respiration, which is independent of ADP phosphorylation. These results suggest that oxidative stress, along with the concomitant phenomenon of sperm DNA fragmentation, negatively affects sperm mitochondrial respiration by an uncoupling between electron transport and ATP synthesis.

At least in principle, a double link exists between mitochondria and oxidative stress. In fact, on the one hand, mitochondria represent one of the ROS generators whereas, on the other hand, they might represent one of the ROS targets.

We eventually investigated the dependence of sperm mitochondrial respiratory efficiency on serum reactive oxygen species, a systemic indicator of oxidative stress (Fig. 3). Blood oxidative stress might also be a consequence of unhealthy lifestyles such as smoking, alcohol abuse, or exposition to chemical or electromagnetic pollution. Interestingly, we found a strong negative correlation between RCR values and a condition of oxidative stress. In fact, RCR values decreased at the increasing of serum reactive oxygen species. Also in this case, only V_4 showed a significantly correlation with radical species.

The analysis of blood oxidative status showed a parallel negative correlation between the levels of serum reactive oxygen species and the percentage of sperm progressive motility. Therefore, the analysis of blood oxidative status could be useful, together with the seminal profile, for the evaluation of sperm quality.

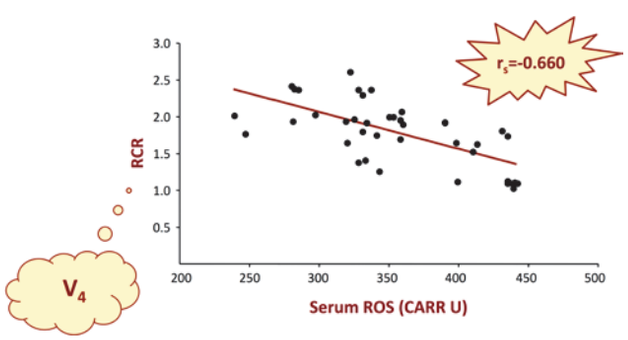

Figure 3: Serum ROMs vs RCR values

5. Varicocele and sperm mitochondrial respiratory efficiency

Varicocele is an enlargement of the veins that drain the testicle. This pathology is one of the main causes of male infertility and can impair sperm quality. Current evidence suggests that oxidative stress is a key element contributing to infertility in men with varicocele. Accordingly to these observations, we found a strong increase in the level of seminal LPO in varicocele samples and a higher incidence of spermatozoa with DNA damage in varicocele patients. Interestingly, in the same patients, we found for the first time an increase in the level of serum ROS, a systemic indicator of oxidative stress (Ferramosca et al., 2015).

The increased levels of ROS in serum and seminal fluid of varicocele patients negatively affected sperm mitochondrial respiration. In fact, when we analyzed the mitochondrial respiratory efficiency by polarography, we found that sperm mitochondria of varicocele patients showed a lower RCR values. In particular, we found a significant decrease in the V_3 values, indicating an impairment of active state of mitochondrial respiration (Ferramosca et al., 2015). We also observed a parallel increase in the percentage of structural defects in the sperm midpiece, corresponding to the sperm mitochondrial sheath.

The defective energy metabolism may play an important role in the impairment of sperm quality in varicocele patients, whose spermatozoa showed a decrease in motility and concentration when compared with control subjects.

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Neuroscience Lab: Section of Cognitive Neuroscience and Psychophysiology

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Abstract

Experimental research in this area focuses, in a section of the laboratory of Neurosciences, on new technologies applied to Cognitive Neuroscience and Psychophysiology. The aim of this paper will be highlighting the link between basic research and innovative multidisciplinary experimentation. According with this perspective the projects developed in the Neuroscience Lab will be briefly described: two devices deposited as European patents, a protocol of olfactory stimulation on EEG and neurodegenerative processing, the research on women with IPV (Intimate Partner Violence disorder), projects on neuroaesthetic (applied in museum learning), a study on the marine soundscape and related brain responses, and an experimental work on an haptic and virtual interfaces and on a comparison of motor imagery training with haptic training of augmented reality, as well as training of handling real objects with grasping affordances. These topics would set out a new view of research, extending beyond the technological interfaces in neurocognitive protocols. Will be described the leitmotif of the research activities carried out over the last five years.

Keywords: Cognitive Neuroscience, Psychophysiology, Olfactory Perception, New Technologies

Neurosciences apply to many fields: most research protocols developed and applied in the Human Anatomy and Neuroscience Lab are focused neurodegenerative diseases (through a murine model); here we will describe the activities which are developed specifically for the DREAM facilities, that, instead, are focused on human Cognitive Neuroscience and Psychophysiology(Boone and Piccinini, 2016; Caramazza, 1992; Stramandinoli et al., 2012). Neurosciences are also applied to robotics, to Artificial Intelligence systems (Anderson, 2003; Artemiadis, 2014; Vernon, 2014), models of pattern recognition, aesthetics, and ergonomics. Also, new paradigms are being defined, which on one hand are interfaced with experimental and "hard" technologies, and on the other hand, they have re-modulated philosophical concepts on meta models, meta-cognition, thought the process and conscience state. They are also contaminated by epigenetics(Eccleston et al., 2007) in a way that creates a sort of "neuro-psychoanalysis" of a nonconscious which becomes collective as an evolutionary product.

The first EEG device present in Laboratory, was acquired by the laboratory with a PRIN on Women and Power. In fact, since 2009, a teaching agreement (which later turned to a research agreement) has been activated with the Women Shelter of Lecce, the so called "Centro Antiviolenza Renata Fonte". We started a research agreement with the Center and the Department of Biological and Environmental Sciences; together we studied, using an electroencephalographic technique called Event Related Potential, or ERP (Luck, 2005), the psychophysiological aspect of women subjected to Intimate Partner violence (IPV), a research that goes on to the present day (Invitto et al., 2014b, 2017d). This was just the first of the many works in the field of neurocognitive sciences that took part at the Laboratory of Human anatomy and Neurosciences in the Department of Biological and Environmental Sciences at University of Salento. Thus, having started from a psychophysiological basis, Invitto

worked on brain Synchronization, by perfecting a non-invasive, brain-stimulating instrument which was linked to physiological rhythms (Synchronization's coil). Such work subsequently got an international patent (Invitto, 2013).
After about one year, thanks to the collaboration with the CNR of Microelectronics and Microsystemics – Lecce Unit, and specifically with Simonetta Capone, Giovanni Montagna and Pietro Aleardo Siciliano (director of the CNR IMM in Lecce), we built a device, which was capable of interfacing an olfactometer with controlled olfactive stimulations to an electroencephalography system (Invitto et al., 2014a).

Fig.1 Experiment on Olfactive perception with MI2014A001344 Requested Patent

Fig.2 Odorous Vials for MI2014A001344

Such device, named VOS EEG (Virtual Olfactory Stimulation) allows the production of controlled olfactive potentials. In this case, the olfactive response is used either for base research and for clinical research, in order to evaluate possible predictive factors of neurodegenerative processes (which showed evidence of an early impairment of olfactive perception ability). This device allowed the development of psychophysiological studies in health subjects and in clinical ease subjects (Invitto et al., 2016b, 2016e, 2017a, 2017c)
In lab's activity, we were involved in an international COST (European Cooperation in Science and Technology), project on "Pain Assessment in Patient with Impairment Cognition, especially in Dementia and Dementia" (coordinator S. Lautenbacher – Bamberg University-German). In COST activity we are studying Pain in Huntington Korea Patients and in other clinical subjects (Defrin et al., 2015).
Also, about Huntington Korea Patients, Invitto developed an interaction with University of Bari (de Tommaso et al., 2017).
For her innovative activities, Invitto was awarded, in December 2015, of the IWIIN (Italian Women Innovators and Inventors) prize, with an ad hoc mention for innovative research. She received, in 2017, an International Award on Innovation: the Gold Innovator Winner 2017. The Prize was awarded by Global Women Inventors and Innovators Network, and by European Women Inventors and Innovators Networks.
The projects relative to innovation started with the mentioned patents and was later developed along with other projects which also received national and international funding. The first project started with the shared request by the Museums of the University of Salento of having innovative interfaces which would value learning objects in the museum (Invitto et al., 2014c) . So, the project was pushed forward with the Cetma and the company Agilex, in order to create products in augmented reality and immersive virtual reality, with interfaces which were analyzed with neuroaesthetics criteria. We choose elements from plancton, which were later developed in the ICT project. In the project, of which Invitto was scientific referent, the Human Neuroscience and Anatomy Laboratory at DiSTeBA, together with Cetma and Agilex

Srl, have built a network- project for museum learning (Ep_Lab).
Other research work, with the use of Augmented and Virtual Reality, are dedicated to the psychophysiological analysis of augmented reality interfaces in entertaining and educational processes (Invitto et al., 2015, 2016c). With the help of EEG techniques and ERP, was analyzed how "real", virtual and augmented interaction are deeply different for our perceptive system, and how this, based on some learning styles, can represent an advantage or a tool which is difficult to interact with, which can slow down behavioural responses and some components of psychophysiological response. In our latest works, we also introduced the comparison between the handling of 3D-printed models(Invitto et al., 2016a).
We are also working on a haptic effector, which we will be developed together with Nabidit Network (CNR Nano-University of Salento) thanks to project Person, with an EEG-interfaced serious game, of Technological Clusters Regione Puglia.
In 2016 a research exchange was developed with The Centre of Robotics and Neural System (CRNS) of University of Plymouth (UK). These exchanges produced research paper (Cangelosi and Invitto, 2017) and a Workshop, in University of Salento, with Prof. Angelo Cangelosi, the Director of the CRNS. The Workshop was titles 'Motion Control and Cognitive Sciences. Two sides of the same coin: Robotics' (fig.3). The meeting focused on theoretical and experimental research based on action and language processing, and on number learning and gestures, that clearly demonstrates the role of embodiment in cognition and language processing. In psychology and neuroscience this evidence constitutes the basis of embodied cognition, also known as grounded cognition (Borghi and Cangelosi, 2014; Caligiore et al., 2013; Pezzulo et al., 2013). In robotics, these studies have important implications for the design of linguistic capabilities in cognitive agents and robots for human-robot communication, and have led to the new interdisciplinary approach of Developmental Robotics (Cangelosi, A; Schlesinger, 2015). During the workshop, we presented examples of developmental robotics models and experimental results from iCub experiments on the embodiment biases in early word acquisition and grammar learning (Morse et al., 2015), experiment on the pointing and finger counting in number learning (De La Cruz et al., 2014) and on mental imagery and rotation (Seepanomwan et al., 2015). The presentation also discussed the implications for the symbol grounding problem and how embodied robots can help addressing the issue of embodied cognition and the grounding of symbol manipulation use on sensorimotor intelligence.

Within a research project on music and brain, we developed another experimentation based on sounds, specifically sounds of the sea, which were recorded with hydrophones; the study were focused on the effects that such sounds have at a cortical level on humans, and thanks to the Zoological Station of Naples, also on animals (Baldascino et al., 2016).
Other studies on music were investigated in Humans (Invitto et al., 2016d, 2017b).

Recently a project was financed through the Cinque Per Mille for University of Salento, on olfactive evocated potentials (developed through VOS EEG patent). This work is based on the cortical analysis of different kinds of olfactive stimulations and the different responses given by healthy individuals and neurodegenerative patients.

Some others clinical experiments were developed through a research agreement with the Department of Basic Medical Science, Neuroscience and Sensory System Department–SMBNOS, within University of Bari (de Tommaso et al., 2014a, 2014b; Vecchio et al., 2016).

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- COST (European Cooperation in Science and Technology) project on Pain Assessment in Patient with Impairment Cognition, especially in Dementia and Dementia (coordinator S. Lautenbacher–Bamberg University-German) http://www.cost-td1005.net//.
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Semiautomatic segmentation of glioblastoma for radiotherapy treatment plannin

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Abstract
During the radiation therapy (RT) process, the treatment is planned and simulated with a treatment planning system (TPS). Contouring identifies the Planning Treatment Volume (PTV), that is the physical RT treatment volume. PTV of Glioblastoma (GB) includes, after expansion, Gross Tumor Volume (GTV, the tumor) and Clinical Target Volume (CTV, tumor plus edema). GlioCAD, a Computer-Assisted Detection software for contouring gliomas in MRI/DTI, was used to delineate GTV. The dataset included the images of 21 patients undergoing RT for GB. For each patient, we co-registered CT-planning images and diagnostic MRI (16 T1-gad, 6 T2 Flair, 13 Flair Fat Sat), which were used for GlioCAD training and validation. CAD outlined the tumor with good accuracy, after ruling out in post-processing some false positives. We identified reliable GTVs, suitable for RT requirements. An evolution of GlioCAD will take into account edema for outlining CTV. The method is promising. Together with a further automatic system for the delineation of organs at risk (OAR) in the brain, the procedure may be helpful for standardization of RT-treatment planning.

Keywords: glioma, glioblastoma, radiotherapy, GTV, contouring, segmentation

1. Introduction

Malignant gliomas are aggressive tumors of the Central Nervous System. The gold standard treatment provides for an initial surgical time followed by radiotherapy and chemotherapy with temozolomide in concomitant and adjuvant setting (Stupp et al. 2005). The prognosis of glioblastoma (GB) multiforme has not improved much over the past ten years since a beneficial in survival with this integrated schedule has been shown. Radiotherapy inevitably induces neurotoxicity and it is normal to consider that limited treatment volumes may consequently reduce it.
However the definition of the optimal radiotherapy treatment volumes in GB remains controversial and may be important to obtain systems that can make the practice of contouring this kind of tumors as homogeneous as possible. Contouring in radiotherapy mainly consists in the identification of two volumes to be treated, when possible, with different doses: the macroscopically visible tumor (GTV, Gross Tumor Volume) and a region (edema) of likely infiltration (CTV, Clinical Target Volume), Fig. 1.
A further margin is added to CTV to create the PTV (Planning Target Volume), which takes into account the uncertainty that may result from positioning errors in the treatment phase.

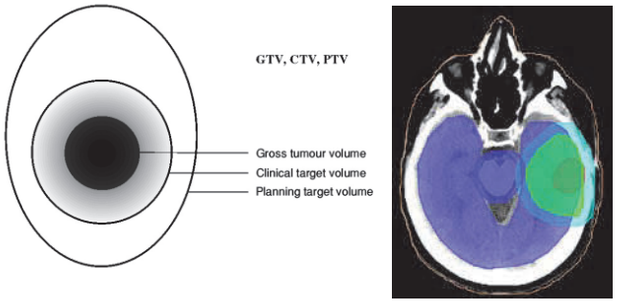

Figure 1. Left: Diagram of the main irradiation volumes (from ICRU 50). Right: an example of manual assessment of GTV, CTV, and PTV (the GBM is in the left brain hemisphere, and the three irradiation volumes are roughly concentric, respectively from the smallest to the largest one).

To improve contouring reliability the radiation oncologist uses centering CT images and diagnostic MR scans.

The treatment volumes in glioblastoma, over the years, have varied in the various cooperative group trials especially differing in margin from the GTV and inclusion of peritumoral edema. For example, the RTOG (The Radiation Therapy Oncology Group) recommends that the initial clinical CTV covers the whole T2 high-intensity areas defined in post-operative MR imaging plus a 2-cm margin, followed by a boost with a field defined by the areas of T1-enhancement (macroscopical residual tumor) and/or the surgical cavity with 2.5-cm margin (Gilbert et al. 2013, 2014). The rationale for peritumoral edema inclusion is the histologically confirmed presence of tumor cells in this area. On the other hand, the EORTC (Organization for Research and Treatment of Cancer) recommends that CTV should include the T1 enhancement area and/or the surgical cavity plus 2-3 cm without the intentional inclusion of T2 areas of edema (Stupp et al. 2005). To support the EORTC protocols there are data that show that the majority of recurrences were within 2 cm away from the primary tumor. Therefore today there is no consensus about the definition of treatment in GB volumes.

It should also be considered that in addition to the heterogeneity in the contouring protocol that can be adopted by each center, there is heterogeneity in contouring due to manual skill, to image interpretation, and to the experience of each radiation oncologist. There are studies assessing the heterogeneity in contouring in which, after surgical resection, some radiation oncologists have surrounded GTV as the entire surgical cavity, which includes both the space of missing tissue filled by fluid and the resection margin, while others have strictly confined the GTV only in the resection margin (see e.g. (Wee et al. 2015)).

In this context, the need for a reproducible and efficient method for delineating the tumor volumes for RT is critical. In particular, it has been demonstrated that MRI is more sensitive than CT in both lesion detection and in the margin delineation of gliomas, see e.g. (TenHaken et al. 1992), which makes MRI modalities the most appropriate for an automatic or semiautomatic GB contouring system and GTV/CTV delineation.

In this preliminary work, GlioCAD (De Nunzio et al. 2011), a CAD (Computer-Assisted Detection) software system for the contouring of cerebral gliomas in MRI/DTI, was used to delineate the GTV treatment volumes in patients with glioblastomas, in order to get quick and operator-independent semi-automatic contouring. The application of GlioCAD was preceded and followed by data processing steps whose purpose was to conform data to the software, and to make the resulting segmentation more appropriate to RT requirements. In this paper, CAD principles and operations are described, the post-processing procedure designed to make the automatically-contoured volumes comply with radiotherapy needs is analyzed, and some segmentation results are shown.

In Section II, the used dataset is described, together with the manual procedure for GTV contouring, which is needed by GlioCAD to initially learn gross tumor delineation, and the software pipeline is illustrated. Section III shows some preliminary results, while the discussion with a small review of the related literature is in Section IV. The conclusions end the paper.

2. Materials and methods.

The dataset consisted of the images of 21 patients treated for GB at the Radiotherapy Unit of Siena (Italy). For each patient we had centering helical CT images and diagnostic MRI, which were coregistered. MR images (16 T1-

gad, 6 T2 Flair, 13 Flair Fat Sat scans) were used to better identify the macroscopic tumor regions (GTV) and the surrounding edema (CTV). The images, comprising manually defined treatment volumes and organs at risk (OAR), were used for the training of GlioCAD, a CAD system for the contouring and volume calculation of cerebral gliomas in conventional MRI and DTI.

The manual contouring procedure had the following steps:

1. We started from the manual segmentations in T1-gad images and used these series to define the macroscopic tumor volume (GTV) corresponding to the area of contrast enhancement; in the case of a post-operative series, the surgical cavity was contoured including any areas with contrast enhancement surrounding the cavity.
2. A margin of 2 cm was added to this volume to obtain the CTV; edema inclusion was verified by exploiting the FLAIR images; in case of imprecise inclusion, we had to apply manual changes to include edema, by comparing the levels of hyperintensity in FLAIR series. If the expansion gave a volume beyond the limits of the cranial theca or beyond natural anatomical barriers (such as bone structures, falx, ventricular system), manual correction was performed.
3. We added a further margin of 0.5 cm to CTV to create the PTV (Planning Target Volume.

When the patients were candidate to receive an additional dose (up to 60-70 Gy), an expansion of about 0.5 cm was given to the initial GTV to create the so-called CTVboost and another 0.5 cm to create a PTVboost; if there are adjacent critical structures these two volumes may coincide (CTVboost = PTVboost).

In conclusion, in each series of images there was a single GTV but possibly more CTV and related PTV (CTV1 with PTV1 and CTV2 with PTV2) which corresponded to the first part of the treatment at lower doses and the subsequent boost – i.e. an additional dose to a limited volume.

The following part describes the application of GlioCAD to automatic GTV contouring, which is depicted in Figs. 2 and 3. The procedure is based on the combined use of T1-gad images and FLAIR images, all of them coregistered with the CT scans.

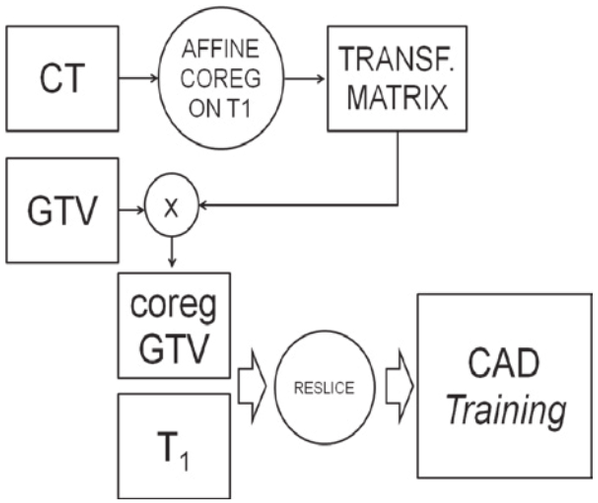

Figure 2. Semiautomatic GTV delineation: preprocessing and CAD training.

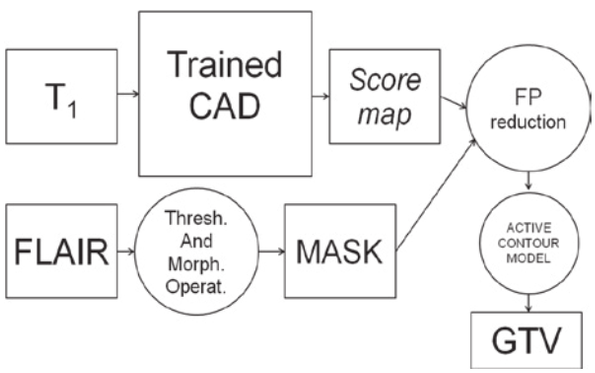

Figure 3. Semiautomatic GTV delineation: Segmentation and post-processing.

GlioCAD is a supervised system, in that it needs an initial set of images where the desired GTV has already been delineated by experts. GlioCAD working scheme (De Nunzio et al. 2011), is based on the calculation of texture features (derived from gray-level histograms, cooccurrence matrices, and run-length matrices) with a sliding-window approach, and on the Fisher Linear Discriminant Analysis as the classification method. After training, the software becomes able to locate and contour by itself the volumes of interest on a different set of images ("validation" set). Accordingly, a preprocessing step followed by a training step is needed. In first place (Fig. 2), the CT images are coregistered (12-parameters affine coregistration) to the corresponding T1-gad,

and the related transformation matrices are used to project the GTV drawn by the radiation oncologist onto the T1-gad images. Coregistrations were performed by *elastix* (http://elastix.isi.uu.nl) (Klein et al. 2010) (Shamonin et al. 2014), which is an open source software tool based on the Insight Segmentation and Registration Toolkit (ITK). *Elastix* consists of a collection of algorithms for (medical) image registration. The modular design of *elastix* allows to quickly configure and compare different registration methods. We used *elastix* command-line interface, which enables automated processing of a large number of images by means of scripting. After coregistration, the T1-gad series with the coregistered GTV was resliced to have homogeneous voxel size in the data set, and was then used to train GlioCAD to recognize and segment the tumor regions in T1-gad.

During segmentation and post-processing (Fig. 3), the trained CAD locates probably cancerous regions in the T1-gad images of the validation set, reducing false positives through a mask obtained from the FLAIR image by thresholding and appropriate morphological operations. An active contour model merges the found regions into a single ROI, smoothing borders and solving inhomogeneity problems. The final identified ROI is used as the (computer calculated) GTV. The quantification of the system accuracy is obtained by the Jaccard coefficient, by comparing the results with the manually defined GTV.

The extension to the CTV will be performed by using again the mask obtained from the FLAIR image, and by considering a 2-cm border added to the GTV by morphological dilation. Finally, to avoid false positives, a descalping mask automatically obtained from the T1-gad images will be applied.

3. Results

Segmentation and accuracy assessment were carried out with Leave One Patient Out (LOPO) Cross Validation: the CAD system was applied in turn to each patient scan (Fig. 3), after training on the remaining images (Fig. 2). In all cases, the CAD outlined the tumor structure with good accuracy. A post-processing step was needed to eliminate some false positives and to make the identified volumes compliant with radiotherapy treatment requirements, with an acceptable hypothesis for the final GTV estimation (Fig. 4). Most of post-processing is automatic, with some manual interventions for verification and refinement. The mean Jaccard coefficient with its standard deviation was $J=0.73\pm0.08$, while the Dice coefficient was 0.83 ± 0.09.

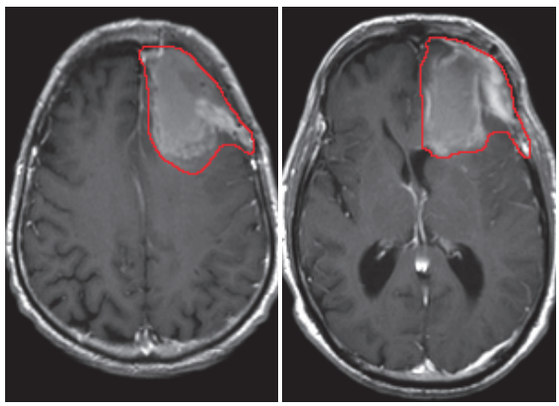

Figure 4. CAD GTV segmentation examples.

4. Discussion

Automatic and semiautomatic segmentation of gliomas (in particular GBs) for volumetric assessment and therapy follow-up have been the subject of an innumerable number of papers (see some recent reviews, such as (Bauer et al. 2013) (Gordillo, Montseny, and Sobrevilla 2013) (Liu et al. 2014) (Simi and Joseph 2015) (Srinivasa Rao and Sreenivasa Reddy 2016)). On the other hand, few works specifically devoted to radiotherapic applications, with a focus on the (semi)automatic contouring of GTV for brain glioma treatment planning, have been published. The subsequent steps, namely automatic expansion to CTV and PTV, have been studied in many papers, some dating back to the nineties, such as (Stroom and Storchi 1997), up to recent works such as (Yan et al. 2014), in which the Authors introduce a method for generating the CTV from GTV using the geodesic distance calculation. These articles will be thoroughly reviewed in a future paper on the expansion to CTV/PTV.

Among the papers concerning (semi)automatic GTV contouring, (Mazzara et al. 2004) compared the effectiveness of knowledge-guided

(KG) and supervised k-Nearest Neighbors (kNN) segmentation for delineating the GTV from MR images of high and low-grade gliomas. The GTV corresponded to the area enclosing several contiguous clusters of enhancing pixels. The average accuracy of the kNN was 56%±6% for 11 cases, whereas that of the KG was 52%±7% for 7 of the 11 cases, compared with the physician's contours. It was observed that kNN and KG are less accurate in contouring GTV in non-enhancing GBs. The study showed severe limitations of the KG-system in handling particular cases such as non-enhancing tumor margins or the presence of non-enhancing cystic necrotic tissues at the center of the tumor. On the other hand, the kNN segmentation method, trained with sample data from MRI slices to segment, lead to more robust segmentation results on all patients.

(Beyer et al. 2006), from the same group, presented a similar and comparative study, extracting GTV with the same two segmentation methods and evaluating the results in terms of predictive dose measurement for therapy planning. It was found that the expert physician reference volume was irradiated within the same level of conformity when using the plans generated from the contours of the segmentation methods. In addition, any uncertainty in the identification of the actual gross tumor volume by the segmentation methods, had small effects when used to generate 3D radiation therapy treatment planning due to the averaging process in the generation of margins used in defining a planning target volume.

(Hori et al. 2010) proposed a method for semi-automated GTV segmentation of GB on brain MR images for radiotherapy planning. Three-dimensional (3D) MR images of 28 GB cases were used. First, a spherical volume of interest (VOI) including the GB was interactively defined. Then, the VOI was transformed to a two-dimensional (2D) image by a spiral-scanning technique. Active contour models were used to delineate an optimal outline of the GB in the transformed 2D image. After inverse transform to the 3D space, a morphological filter was applied to smooth the shape of the 3D segmented region. The computer output was compared with the manually segmented regions by the Jaccard similarity coefficient (JSC) and the True Segmentation Coefficient (TSC), giving on average 74.2±9.8% and 84.1±7.1%, respectively. This paper is written in Japanese.

The Expectation Maximization (EM) algorithm, applied on a Gaussian mixture model consisting of pure superpositions of Gaussian distributions, was employed in (Simon et al. 2012) to delineate the Apparent Diffusion Coefficient (ADC) areas of high and low proliferation in heterogeneous gliomas from predefined manual GTVs on 2D DWI slices. The EM was initialized manually from the contoured ROIs. The result was a reproducible quantification in regions of tissue inhomogeneity. Reproducibility of this approach was evaluated in 10 patients with glioma. Moreover, an automatic initialization approach that completely removes user-induced variability was introduced.

In (Unkelbach et al. 2014) brain segmentation (normal tissues vs tumor) was obtained by an algorithm based on EM, which uses a probabilistic normal tissue atlas as spatial prior. For every voxel, it estimates the posterior probability for three normal tissue classes (white matter, gray matter, and CSF), as well as the lesion outlines on T1 post gadolinium and T2-FLAIR. It then uses the Fisher-Kolmogorov glioma growth model to assess lesion infiltrations in normal appearing regions of the brain. In the paper, the need for reliable segmentation of anatomical boundaries such as the *falx cerebri* and the *tentorium cerebella* is put in evidence. The target volume for radiotherapy planning is defined as an isoline of the simulated tumor cell density. Dice metrics are given between manual and model-derived CTV volumes (values range from 0.74 to 0.84).

(Dittmann et al. 2013) considers that conventional imaging modalities reveal only the central part of the tumor with a high cellular density, but fail to detect microscopic tumor cell infiltrations. Mathematical models can be used to integrate known growth characteristics of gliomas into the target delineation process. In the paper, the Authors demonstrate the use of diffusion tensor imaging (DTI) for simulating anisotropic cell migration in a glioma growth model that is based on the Fisher-Kolmogorov (FK) equation. For a clinical application of the model, it is crucial to develop a detailed understanding of its behavior, capabilities, and limitations. For that purpose, the Authors perform a

retrospective analysis of glioblastoma patients. It was found that, depending on the location of the tumor relative to major fiber tracts, DTI can have significant influence on the shape of the radiotherapy target volume.

Finally, the work described in (Stretton et al. 2013) is worth noting and is related to (Dittmann et al. 2013). Tumor growth models based on the FK equation are employed again, but replacing DTI (costly and not always available) with an isotropic diffusion map or an anisotropic high-resolution DTI atlas formed by averaging DTIs of multiple patients. Three metrics are used to quantify the impact of replacing the patient DTI: the shape of the simulated glioma, the estimation of the tumor growth parameters, and the prediction performance on clinical cases.

The preceding review shows that the literature does not offer a large quantity of papers devoted to the specific subject of GTV automatic or semiautomatic contouring. Direct comparison of our results is possible only with (Mazzara et al. 2004) and (Hori et al. 2010).

In (Mazzara et al. 2004) the ground truth for accuracy assessment is obtained by multiple contours drawn by three physicians, and the probability that a given pixel is properly classified as part of the tumor (its "weight") is determined by the number of times that this pixel was included in the outlines prepared by the radiation oncologists. Accuracy for the computer segmentation is then defined as the ratio of the total sum of weights contained within the computer segmentation volume to the total weights generated from the volumes produced by the physicians. This measure is a kind of probabilistic true-positive rate (i.e. sensitivity). Observing that by definition the Jaccard coefficient is always lower than or equal to sensitivity, our method clearly outperforms their results. As to (Hori et al. 2010), the absence of an English version of the paper makes a detailed comparison difficult. As to segmentation quality, our results in terms of Jaccard coefficient look similar.

5. Conclusions

The proposed method is promising, although still under development and not fully automatic. In the next future, it will be tested on a larger set of images, and the procedure will be made as automatic as possible. The following step will be CTV/PTV automatic delineation, which is currently work in progress. Together with the use of automatic or semiautomatic systems for OAR delineation, for which the literature already presents various solutions often based on atlas-driven approaches, e.g. (Isambert et al. 2008) (Daisne and Blumhofer 2013) (Consona et al. 2014), the procedure may be of help to optimize radiation-treatment planning in patients with GB, in particular to make the process of contouring as homogeneous as possible without operator-dependent variability and to obtain limited treatment volumes in order to reduce RT-related neurotoxicity.

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Transformed Follicular Lymphoma: Not all fit in one

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Abstract
Follicular Lymphoma (FL) is the second most frequent non-Hodgkin lymphoma accounting for approximately 10-20% of all lymphomas in Western Countries. Histologic transformation (HT) is a frequent event in the clinical course of patients with indolent lymphoma that is often accompanied by a dramatic change in the clinical features of the disease towards a more aggressive course. Although the transformation of Follicular Lymphoma (tFL) was described several decades ago, there is a strong need for a better understanding of both the dynamics of the tumor clonal evolution and the genetic events leading to ()transformation. In addition, the management of patients with tFL is challenged by the heterogeneity of the previous treatments. The present review describes the state of art of tFL, outlining recent advances in the understanding of genetic basis and the evolutionary process governing the initiation and persistence of tumor evolution. It will be also addressed the key questions pending on this incurable disease, such as a lack of a standard therapeutic strategy for tFL patients as well as its outcome in the Rituximab (R) era.

Keywords: Non Hodgkin Lymphoma, Follicular Lymphoma, Transformed Follicular Lymphoma, immunochemotherapy

Introduction

Follicular Lymphoma is defined by the World Health Organization (WHO) classification (Swerdllow SH, Campo E 2008) as a "neoplasm composed of follicle center (germinal center – GC) B cells, typically both centrocytes and centroblasts which usually has at least a partially follicular pattern". FL arises then by a malignant transformation of the normal germinal center B cells and therefore carries the characteristics of centrocytic/centroblastic morphology.
The disease is usually characterized by an indolent clinical course, excellent response to initial therapy with frequent relapses and shorter duration responses to salvage therapy.
At present, thanks to the advances in the treatment of FL, the disease is moving from an incurable to curable one. Regarding the management chemoimmunotherapy – usually followed by Rituximab maintenance – is the standard of care; whereas patients with nonbulky or asymptomatic disease may be treated with Rituximab monotherapy or simply observed (Jonathan W Friedberg et al. 2009). Although many asymptomatic patients with low-volume disease may not require early therapy and can be observed, the majority of patients will lastly experience disease progression and will need therapy during the course of the disease. In particular, some patients have long-term remission lasting years, others have a rapidly progression of the disease and develop treatment resistance and/or transformation to aggressive lymphoma (Link et al. 2013; S. M. Smith 2013). Priorities in goals of care include avoiding relapses, transformation to aggressive subtypes and death.

In the past, approximately 25% of cases transform to aggressive disease, mostly Diffuse Large B-cell Lymphoma (DLBCL),with a very poor prognosis (Montoto et al. 2007). The histologic changes seen in patients with HT are, in the great majority of cases, accompanied by a change in the clinical features of the disease towards a more aggressive course (Montoto 2015).

More recently, contradictory data are emerging with respect to the impact of initial treatment on the risk of transformation (Montoto 2015), In particular, the adoption of Rituximab in first line therapy seems associated with a lower risk of transformation. Therefore, decisions on the management of tFL patients come from extrapolation data of retrospective studies or from prospective trials in DLBCL, as this is the commonest transformed lymphoma.

The present review will be focused on the state of art of tFL and will try to address pending questions about this condition.

Definition of tFL

Definition of tFL is still a great challenge as it varies among different series. Over the past years, definition of transformation have been largely varying from "*an histological features of DLBCL as opposed to cytologic progression with an increase in the proportion of large cells (from grade 1/2 to grade 3 FL)*" (Montoto et al. 2007) to "*refractory/recurrent disease with either clinical or pathologic diagnosis of transformed lymphoma*" (Link et al. 2013); passing through other definitions. The main issue in considering a unique definition is the fact that transformation is diagnosed in different series based on cytological samples, on histologic samples or in some cases on clinical grounds alone. Indeed diagnosis should be based on biopsy or adopting clinical criteria in the cases in which it is not possible to obtain a biopsy, either because of the poor performance status of the patient or because of progression of the disease in inaccessible areas (Montoto and Fitzgibbon 2011). In 2008, Al-Tourah et al. (Al-Tourah et al. 2008) published clinical criteria to define transformation: a rapid discordant lymphadenopathy growth, unusual sites of extranodal involvements, a sudden rise in the LDH level, hypercalcemia, or presence of new B-symptoms.

Incidence, Prognosis and Outcome of tFL

Historically, transformation was largely considered a catastrophic event. Although the clinical course of FL patients may span more than ten years, transformation occurrence heralds a change from an indolent to an aggressive disease course, and is associated with major morbidity and mortality (14). In particular, most of the studies have reported a poor prognosis after transformation, with a median duration of survival generally ranging from 2.5 months to 2 years, with most deaths being due to lymphoma (5,7,12,13,15–21).

The incidence of HT have wavered over the past several decades, due to the adoption of different diagnostic methods, definition of transformation and duration of follow-up. Thus, the considerable variability in the incidence of tFL reported in literature may be explained by the heterogeneity in the definition of transformation, population included and diagnostic tools.

The clinical significance of transformation was seen in 325 FL patients from the St Bartholomew's Hospital of London, in which the risk of transformation by 10 years was 28% and the median survival after transformation was 1.2 years. Patients with tFL had a significantly shorter OS and a shorter survival from progression compared to others (Montoto et al. 2007).

Al-Tourah et al. analyzed the incidence of HT in a population based-study of 600 patients. The annual risk of transformation was estimated to be 3% per year, and the median survival after transformation was 1.7 years (7).

In another recent study from the University of Iowa/Mayo Clinic (Link et al. 2013), the cumulative risk of transformation and death without transformation (competing risk) increased steadily over time up through 5 years of follow-up and then appeared to slow, with only four transformations observed beyond 5 years from diagnosis. Transformation rate at 5 years was highest in patients who were initially observed and lowest in patients who initially received Rituximab monotherapy (Link et al. 2013).

However, in a group of 107 patients with advanced FL and low tumor burden registered in

the F2 database and managed with a W&W policy, the 5-year risk of transformation was quite low. After a median follow-up of 64 months, five patients experienced transformation to aggressive non-Hodgkin lymphoma, two during the W&W no treatment period and three after progression, with an estimated rate of less than 1% per year (Solal-Céligny et al. 2012).

Wagner-Johnston et al. have investigated the incidence, prognostic features, and outcomes associated with tFL among 2652 patients with FL prospectively enrolled in the US National LymphoCare Study. At a median follow-up of 6.8 years, 14.3% of patients underwent transformation; patients who were treated at diagnosis had a reduced risk of transformation as well as maintenance Rituximab was associated with reduced transformation risk. The median OS post transformation was 5 years (Wagner-Johnston et al. 2015).

The clinical and laboratory findings with better Overall Survival (OS) at the time of transformation include normal LDH levels, limited disease extent, good performance status, absence of B symptoms, fewer number of previous relapses, transformation after expectant management, having had no prior CR, or having had no response to salvage chemotherapy (5,7,12,13).

Despite the overall poor outcome of tFL patients, Yuen et al., identified a subset of patients having a relatively good outcome (Yuen et al. 1995). Limited extent of disease, attainment of Complete Remission (CR) to treatment given at the time of transformation and no prior therapy had a particularly favorable prognosis. In addition, patients who achieved CR after transformation had a better OS than those with advanced stage disease (108 vs 18 months) (Yuen et al. 1995). The impact of limited disease on tFL patients' prognosis was also seen by Bastion et al., (Bastion et al. 1997) and Al-Tourah et al.,(Al-Tourah et al. 2008). This latter group shown that the 5-year OS was 66% for patients with a limited transformation compared to 19% for those with advanced-stage at transformation in a significant way (Al-Tourah et al. 2008).

Only slight improvement were observed in recent studies, mostly showing the shorter OS of tFL patients in comparison to non-transformed FL patients. This adverse effect of transformation on survival was clearly illustrated by Al-Tourah et al., where the 10-year OS for non-transformed FL patients was 75%, whereas was only 36% for tFL patients were alive 10 years from the time of their initial FL diagnosis (Al-Tourah et al. 2008). Similar behaviors were also seen in other studies (Montoto et al. 2007).

Importantly, most of the patients in the reported studies received therapies not incorporating Rituximab. However, differences in patients' series, treatment and outcome is not a good condition to take advantage of; a lot of work is still needed to find out standard clinical criteria. Table 1 summarizes transformation risk in recent series.

Table 1. *Outcome of tFL patients in recent series*

Studies	Transformation risk
Montoto et al., 2007	**17%** at 5 years (100% biopsy proven) **1.9 RR in W&W**
Al-Tourah et al., 2008	**18%** at 5 years (63% biopsy proven) 30% at 10 years **(18% Doxo vs 30% Alk P=0.001)**
Link et al., 2008	**10.7%** at 5 years (85% biopsy proven) **14.4% in W&W** **3.2% in Rituximab**
Wagner et al., 2005	**12.8%** at 5 years (39% biopsy) **13.4% Rituximab chemotherapy** **18.3% NonRituximab chemotherapy**
Sarkozi et al., 2016	**4.1%** at 6 years (100% biopsy proven)

Can we assess any improvement in the outcome of tFL in the Rituximab era?

Whether the addition of Rituximab in intial treatment modifies the outcome of tFL patients, still needs to be addressed. However, over the last 5 years, several studies have been suggesting that the outcome of tFL patients has improve in the Rituximab era (Conconi et al. 2012; Ban-Hoefen et al. 2013; Lerch et al. 2015; Link et al. 2013; Guirguis et al. 2014).

Very recently Sarkozy and all analyzed risk factors, incidence and outcome of HT at first recurrence in the PRIMA patient cohort: after 6 years after a chemoimmunotherapy induction, the cumulative incidence of HT was 4.1% (Sarkozy et al. 2016).

Literature suggests that patients treated with Rituximab-containing chemotherapy achieve a longer OS compared with retrospective cohorts of patients treated with chemotherapy alone (Link et al. 2013; Bastion et al. 1997). In contrast, the most recent trial comparing a W&W

approach vs R did not show any differences in terms of risk of transformation (Ardeshna et al. 2014). As commented by the same authors, in the most of the reported series, the majority of patients have received chemotherapy prior to the diagnosis of HT and have advanced stage at the time of transformation so the better outcome cannot be attributed to an earlier identification of transformation leading to a better risk population (Montoto 2015).

Controversial results were also found in whether prior treatment with Rituximab has a good effect on the outcome after transformation. In a recent study, Lerch et al. (Lerch et al. 2015), showed that the treatment with Rituximab before the diagnosis of tFL was not correlated with a worse outcome in those patients. In contrast, patients with relapsed DLBCL treated with Rituximab have a significantly worse prognosis at progression (Montoto 2015). Other two studies demonstrated that prior R treatment did not result in a worse outcome in tFL patients who received high dose therapy with autologous stem cell rescue (HDT-ASCR) (Ban-Hoefen et al. 2013; Madsen et al. 2015). Patients who have previously received CHOP (Rituximab, Cyclophosphamide, Hydroxydaunorubicin, Vincistrine [Oncovin], Prednisone) chemotherapy, with or without R, are frequently treated with second line regimens for DLBCL (Montoto 2015).

The role of Rituximab maintenance in DLBCL patients is also still unconcluded. In a recent study, R Maintenance was associated with reduced transformation risk. The median OS post transformation was 5 years, suggesting an improved outcomes for transformation in the modern era (Wagner-Johnston et al. 2015). Habermann et al., (Habermann et al. 2006) showed that addition of maintenance with R did not improve the outcome of DLBCL patients treated with R-CHOP. Other two studies showed that R maintenance after HDT-ASCR in DLBCL patients was not associated with a better outcome (Haioun et al. 2009; Gisselbrecht et al. 2012).

Based on heterogeneity of the treatment used before diagnosis of HT, it is a challenge to pull out any co clusions about specific regimen.

Pathology and biology of tFL

Although the FL transformation to DLBCL was described several decades ago, there is a strong need for a better understanding of both the dynamics of tumor clonal evolution and the genetic events that are responsible for transformation.

The pathogenesis of FL is best explained by a unifying hypothesis that takes into account genetic alterations harbored by the neoplastic B cells and an immunological model that suggests prominent crosstalk between the tumor cells and non-neoplastic immune cells in the tumor microenvironment, which include T cells, macrophages, follicular dendritic cells and stromal elements (de Jong and de Boer 2009; de Jong 2005; I S Lossos 2005).

It is well known that the translocation t(14;18) characteristic of most cases of FL leads to constitutive B-cell Lymphoma 2 (BCL2) protein expression and is a critical early event in the development of FL (Izidore S. Lossos and Gascoyne 2011). These cells then slowly proliferate, but do not die by apoptosis, and they can acquire genetic alterations. This condition can include chromosomal alterations that result from the localization in the GC (Küppers 2005). Others are driver mutations and alterations that provide the malignant cells with a growth advantage including gains, losses of chromosomal material and even balanced translocations involving the activation of dominant oncogenes, such as MYC (Izidore S. Lossos and Gascoyne 2011).

Recent studies have shown divergent pathways of disease progression and transformation in FL.

Recent whole-exome studies have highlighted that low-grade FL at diagnosis frequently shows some "driver" mutations not found in the transformed clone (Okosun et al. 2014; Pasqualucci et al. 2014; Carlotti et al. 2009). Some of these data derived from genetic methods, and suggest that FL may evolve as a "non linear" transformation (Okosun et al. 2014; Pasqualucci et al. 2014; Bouska et al. 2014; Wartenberg et al. 2013; Green et al. 2013; Eide et al. 2010; Carlotti et al. 2009; Ruminy et al. 2008; Halldórsdóttir et al. 2008) , in which the clone detected at transformation is more closely

related to a common progenitor than the clone predominating at the time (or site) of prior sampling (Casulo, Burack, and Friedberg 2015). The clonal evolution models to tFL were defined by investigating genomic alterations that are present in the dominant clone of both pre- and post- specimens ("Shared lesions"), and contrasting them to those that are present exclusively in the FL or tFL biopsy ("phase-specific lesions"). This analysis allowed to discriminate between i. a linear model, in which the tFL dominant clone will maintain all lesions present in the original FL clone, in combination with additional tFL-acquired alterations; ii. a divergent evolution model, where there are lesions that are unique to the dominant clone of the FL or the tFL, in addition to the set of shared alterations (Pasqualucci et al. 2014).

Thus, data mostly supported a divergent evolution model in a significant proportion of patients undergoing transformation, whereby FL and tFL arise from a common mutated ancestor through the independent acquisition of distinct lesions (Pasqualucci et al. 2014). Most FL and tFL derive from a common mutated precursor cell through divergent clonal evolution. There are some shared molecular determinants such as chromatin modification and apoptosis and some tFL specific determinants such as cell cycle, proliferation and DNA damage response.
Interestingly, the most commonly affected genes in both FL and tFL where those encoding for histone/chromatin modification enzymes, including methyltransferases and acetyltransferases (Pasqualucci et al. 2014). These early lesions in FL generally affect epigenetic regulators (genes controlling chromatin structure), including the H3H4 trimethyl-transferase *MLL2* mutation, never lost at transformation, EZH2 and the acetyltransferases *CREBBP* and *EP300* (Okosun et al. 2014; Pasqualucci et al. 2014; Morin et al. 2010; Morin et al. 2011).
Other frequent dysregulation in both FL and tFL was represented by programmed cell death genes, in particular BCL2 translocations, and thus presumably in the common ancestor clone (Pasqualucci et al. 2014).
Among tFL specific determinants there were alterations of cell cycle control, through mutation or deletion of cyclin-dependent kinase 2A/B (CDKN2A/B). These latter are two tumor suppressor genes, whose protein products p14-ARF, p16-INK4A and p15-INK4B, are important for negative regulation of cell cycle G1 progression and stabilization of the tumor suppressor p53 (Pasqualucci et al. 2014; Sherr 2004).
Alterations in *Myc* (Okosun et al. 2014; Pasqualucci et al. 2014), as well as DNA damage response, through losses of genes associated with regulation of the immune response, were also consistent in tFL only. Among the mutations that affect the immune response, recent studies found the entire HLA class I locus, specifically in β-2-microglobulin (B2M) and CD58 (Pasqualucci et al. 2014; Bouska et al. 2014; Morin et al. 2010).

Although a large number of prognostic markers have been implicated as contributing to survival in FL, only a handful have specifically examined the role of biological factors impacting risk of transformation.
It is becoming evident that phenotype variations related to genetics events (MYC, BCL2, p16, p53) should be routinely identified. FISH analysis could also be required to identify genetic alterations in MYC, BCL2 and BCL6.
The incidence of CD30 expression in tFL has also been reported to be 20% in a recent retrospective series of cases and CD30 expression should be also routinely identified by IHQ.
In summary, there is not a single mechanism driving transformation from FL to DLBCL (Okosun et al. 2014; Pasqualucci et al. 2014; Bouska et al. 2014; Andrew J. Davies et al. 2007). Rather, there are several mechanisms involved in transformation.

Treatment of tFL
Optimal treatment strategies for tFL still represents an unmet need. Unfortunately, most clinical trials exclude patients with tFL and there are no randomized studies in the modern era, with the result that level of evidence is very limited.
In historic series, as described before, the outcome of tFL patients was very poor, with a median OS of approximately 1 or 2 years (Montoto and Fitzgibbon 2011). However, the majority of published studies were conducted in the pre-Rituximab era, making difficult to draw any conclusion on the current scenario.

In the study conducted by Lynk and colleagues in 60 out of 631 patients with biopsy-proven tFL, the median OS was 50 months with an OS rate of 73% at 5 years after treatment with R-CHOP chemotherapy (Link et al. 2013). Survival was similarly in the National Comprehensive Cancer Network (NCCN) database study, with a median OS around 5 years in 118 biopsy confirmed tFL patients, was (Ban-Hoefen et al. 2013). Similar results were seen in early-stage FL experiencing tFL, with a 3-year OS of 44% (Bains et al. 2013). The estimated median OS for the patients with a histological diagnosis of HT from the PRIMA trial was 3.8 years after a median 6 yea follow up (Sarkozy et al. 2016).

As response after conventional chemotherapy, high-dose therapy (HDT) and autologous stem cell transplantation (ASCT) has been studied by several research groups (Witzig et al. 2002; Schouten et al. 1989; Freedman et al. 1991; Foran et al. 1998; Berglund et al. 2000; Chen et al. 2001; Williams et al. 2001; J W Friedberg et al. 1999; Andreadis et al. 2005; Sabloff et al. 2007; Ramadan KM, Connors JM 2008).

Of historical relevance, the efficacy of ASCT was shown in tFL patients in old phase 2 and transplant registry series prior to the incorporation of immunotherapy (Wang and Hou 2010; Montoto and Fitzgibbon 2011). In particular, 40% of patients experienced long-term benefit, with survival rates similar to patients with relapsed aggressive lymphoma receiving the same treatment (Williams et al. 2001). However, most of the transplant studies are based on only small retrospective series of 20 to 50 patients (Hamadani et al. 2008; Williams et al. 2001; Chen et al. 2001; J W Friedberg et al. 1999; Sabloff et al. 2007). The role of ASCT has been further investigated in the Rituximab era, and so more relevant to current practice with the result that the addition of the antibody has improved the outcomes of tFL patients practice.

The Canadian Bone Marrow Transplant group (CBMTG) analyzed 172 patients with tFL , 53 treated with rituximab containing chemotherapy alone and 97 underwent instead ASCT. This latter approach improved OS and Progression Free Survival (PFS) of patients over Rituximab-containing chemotherapy regimens, although the difference was modest (Villa et al. 2013). Other groups observed that patients who were Rituximab naïve prior to ASCT, seemed to achieve better results than those with prior Rituximab exposure (Wirk et al. 2014; Kuruvilla et al. 2015; Ban-Hoefen et al. 2012; Gisselbrecht et al. 2010; Villa et al. 2013; Madsen et al. 2015) paralleling the observation in de novo DLBCL patients undergoing ASCT (Gisselbrecht et al. 2010). In addition, patients with early tFL performed significantly better in terms of OS, compared to those with late tFL(Link et al. 2013). ASCT had similarly outcomes in the large NCCN database, with an OS of 83% being superior compared to chemotherapy alone or ASCT without the incorporation of monoclonal antibodies (S. D. Smith et al. 2009; Villa et al. 2013).

Allogeneic transplantation in tFL has been less well studied, with small numbers of patients in mostly retrospective series. Some of these studies showed significantly inferior results than ASCT (Ramadan KM, Connors JM 2008), most probably due to the higher treatment related mortality (TRM) associated with the allogenic approach (35% versus 10% at 5 years). In contrast, the risk of disease relapse at 5 years was tendentially lower.

For relapses after ASCT, further salvage therapy with allogeneic transplantation seemed to improve the outcome of tFL regardless of the significant transplant related mortality (Ratanatharathorn et al. 1994; Doocey et al. 2005).

Radioimmunotherapy (RIT) has been proposed as primary treatment for tFL. In particular, radioactive nucleotide antibodies yttrium Y^{90} ibritumomab (Zevalin) and iodine I^{131} tositumomab (Bexxar), have shown some anti-lymphoma activity in tFL patients (A. J. Davies et al. 2004; Kaminski et al. 2001; Vose et al. 2000; Witzig et al. 2002). The overall response rates of RIT was 51%, ranging from 39% and 79%, with half of the responsers achieving complete remissions (CR) (Wang and Hou 2010). (Izidore S. Lossos and Gascoyne 2011). In the largest of these studies, Zelenetz et al., (Zelenetz AD, Saleh M, Vose J 2002) evaluated 71 patients from several I^{131} tositumomab studies, and showed a median duration of response of 36 months in responding patients. Although this approach seemed less effective in patients with bulky tumor burden and patients who

have previously received radiotherapy, it might be considered especially in patients not qualifying for more aggressive approaches giving that CR patients have shown prolonged response, often longer than 1 year (Izidore S. Lossos and Gascoyne 2011).
Moreover, an additional area of interest involves the integration of RIT with HDT and transplant in tFL patients, that has the potential to improve disease control, with similar toxicities compare to HDT alone (Krishnan et al. 2008; Wondergem et al. 2012; Reddy and Savani 2011; Mei et al. 2014).

Recently, novel agents have been investigated in tFL. In a phase 2 study, Lenalidomide showed an overall response rate of 57%, with median response duration of over 1 year in tFL patients (Czuczman et al. 2011). Specific inhibitors, targeting Aurora A kinase (alisertib) (Jonathan W. Friedberg et al. 2014), Bruton tyrosine kinase (ibrutinib) (Aalipour and Advani 2013), the δ isoform of phosphatidylinositol 3-kinase (idelalisib) (Gopal et al. 2014; Burger and Okkenhaug 2014) and the BCL2 protein (GDC-0199/ABT199) (Seymour JF et al. 2013), are currently being investigated in both indolent and aggressive lymphomas. These novel agents seem to have a significant impact on the outcome of tFL patients.
Recent efforts are focusing on the immune tolerance towards lymphoma cells as an alternative therapeutic approach. Pidilizumab is currently used as a monoclonal antibody to Programmed death-1 (PD-1), a member of the B7 receptor family that represents an important immune checkpoint regulator. Its efficacy has been recently shown in ASCT patients with DLBCL, including a subset with tFL (Armand et al. 2013).

Conclusions

HT is expected as a relatively frequent event in the clinical course of patients with indolent lymphoma. However, The incidence of HT varies enormously amongst different series, pending on the definition of HT, which is different in different studies., and different treatment approaches of the FL. Based on the available published studies, mostly derived from small retrospective studies, there is still not any standard therapeutic strategy for tFL patients: treatments used are different in different reports and relationship between treatment and outcome does not emerge very well from the literature. The fact that the risk of transformation is rarely an end-point in prospective studies underlined a great obstacle in this field, so there is no clear evidence that the initial management has an impact on the subsequent risk of transformation.
In conclusion, There is need for further studies aiming to provide an answer to pending question, including i. Definition of tFL; ii. Assessement of risk of transformation in non-treated patients; iii. Potential role of a FL stem cell or repopulating cell as a potential cell of origin contributing to histologic transformation; iv. History of clonal evolution; v. Molecular determinants; vi. Response to salvage therapy of tFL; vii. Outcome of tFL in the Rituximab era.
In addition, other several questions should be addressed, such as whether De novo tFL is the same or a different disease; whether FL3b is different from tFL and if there are differences between tFL at first or subsequent relapse.

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The cost-effectiveness of ambulatory vs laboratory based sleep services management of OSA

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Abstract
The Obstructive Sleep Apnea (OSA) is becoming a significant problem nowadays because it is associated with an increasing cardio-metabolic mortality. This disorder is increasing dramatically in the western world because it related to obesity. The cost-effectiveness ratio of OSA's diagnostic and therapeutic management is a strategic issue for health policy, which faces with recent international discussion involving the Western Health Systems. It has been shown that costs for simplified models used for diagnosis and outpatient care for OSA are minimal due to a more and more reduction of expenditure for both equipment and medical staff. This lack of investments and the utilization of portable and simplified monitoring devices could lead to incorrect diagnosis. This short review aims to offer discussion topics on the proper diagnosis and treatment of OSA in view of epidemiological / economic factors and results in terms of costs and social benefit of the disease.

Keywords: Obstructive Sleep Apnea (**OSA**); Epworth Sleepiness Scale **(ESS)**,cost-effectiveness (**CE**); Health Technology Assessment (**HTA**), Affordable Care Act (**ACA**); Teleservice **(TelaDoc);** Home sleep testing (**HST**); Continuous Positive Airway Pressure (**CPAP**); telemedicine interventions (**IT**)

Laboratory vs portable monitoring for the management of OSA

The growing concern that have been manifesting toward Obstructive Sleep Apnea (OSA), especially in the Western world, has led the focus on the more and more clear correlation between OSA and the reduced quality of life as well as the increment of cardiovascular and metabolic mortality (Edinger et al. 2016, 237-247) . The major OSA risk factor is obesity that is growing as the incidence of OSA (Romero-Corral et al. 2010, 711-719)**.** This condition often is underrecognized in hospitalized obese patients. A recent study, (Sharma et al. 2015, 717-723) has showed **to a** total of 636 obese patients admitted to medical center were classified as high risk Within 4 of discharge, 87% were shown to have OSA. The study shows significant under-recognition of OSA in obese hospitalized patients.

OSA (Epstein et al. 2009, 263-276) is characterized by daytime sleepiness, h**u**abitual snoring and at least 5 respiratory-obstructive events per hour of sleep, or >15 apneic events per hour in absence of symptoms. Other physical characteristics are highlighted in the pre-clinical test for assessing the probability of OSA, that include: BMI> 30, neck circumference >43 cm in men and 41 in women, daytime hypersomnolence measured with a self-assessment questionnaire, called the Epworth Sleepiness Scale (ESS) in which the cut-off is greater than 10.American Academy of Sleep Medicine guidelines suggests that portable monitors (nocturnal cardiorespiratory monitoring MCRN) may underestimate the seriousness of the ipopneic events compared to a complete polysomnography - Level 1 - that has been performed in a sleep laboratory The exponential increment of the OSA incidence, in particular in the general population from 40 to 70 years old, led to an unmet need to simplify

the OSA diagnosis using simplified diagnostic tests and to improve the treatment. In the past, the OSA diagnosis was performed into the sleep laboratory situated in the hospital, using polysomnography test, which required complex equipment and updated medical staff. Pietzsch et al. have performed a cost-utility study for the diagnosis of OSA. (Pietzsch et al. 2011, 695-709). This research group have assessed the cost-benefit ratio by comparing the three most used diagnostic / therapeutic strategies: a) overnight PSG along with a manual titration of the CPAP working pressure, in the sleep laboratory, b) split-night PSG along with manual titration of the CPAP working pressure; c) unattended portable monitoring (HST) with subsequent treatment with APAP (auto-titrating CPAP).

Another, recent study (Ayas, Pack et Marra 2011, 691-692) has considered 191 suspected OSA patients tested in advance using a preclinical test. Among them, more than half (56.5%) have manifested obstructive sleep apnea. Diagnosis of OSA was missed in 5.8% patients only, due to the lack of an accurate diagnosis from a specialist doctor of a sleep apnea.

The probability to obtain an accurate diagnosis using pre-clinical tests seems not to be influenced by the presence / absence of a specialist doctor in accordance with the severity of the disease. The authors concluded that OSA could be reliably identified using HST test regardless of pre-clinical test.

The increment of the need in diagnostic tools, (Aurora et al. 2016, 725-730; Huang et Rosenthal 2014, 1376-1379) have led the US to organize a home care model, a simplified Home Sleep Testing (HST) service company, refundable by the US insurance agencies, that deals with both diagnosis and therapeutic care of OSA patients. The services company called (Affordable Care Act) ACA, aims to provide high quality healthcare to OSA patients.

The ACA is gearing up towards a diagnostic model that focuses on the doctor-patient relationship (PCMH). In this home care model, the company puts at the center of this relationship a network of healthcare services where the primary cares are subsequently and rapidly integrated after being diagnosed. Basically, once the diagnosis and the treatment are determined, the Agency rapidly provides a home care technical support. This HST model is not inferior to other diagnostic / therapeutic models. This home care diagnosis and treatment is performed by health care professionals along with a consultation with a sleep specialist once. This approach reduces the economic costs of the medical staff and simplifies the delivery steps in providing the therapeutic equipment at home. This model results in a diagnostic improvement and in the increment of undiagnosed cases of OSA (Antic et al. 2009, 501-508; Davis, Abrams et Stremikis 2011, 1201-1203).

CPAP treatment: clinical and compliance

The treatment with the Continuous Positive Airway Pressure (CPAP) devices is the preferred treatment for severe OSA (Kushida et al. 2008, 157-171). The CPAP device consists of a nasal mask and / or oro-nasal through which an airflow is generated at continuous positive pressure by a compressed air blower system at low pressure. This established controlled pressure is designed to overcome the forces exerted by the soft tissues surrounding the upper airways. Because of this, treatment with CPAP is referred to as a 'pneumatic splint' (Donovan et al. 2015, 1323-1342).

Same studies have shown the benefits of treatment with CPAP in OSA as a mainstay of the treatment of symptoms such as snoring, daytime sleepiness, improvement of quality of life and of sleep as well as secondary depressive disorders (Cruz, Drummond et Winck 2012, 361-66; Donovan et al. 2015, 1323-42). Furthermore, it was shown that the treatment with CPAP reduces the risk of cardiovascular complications such as, stroke, heart failure, chronic ischemic heart disease and arterial hypertension (Barbé et al. 2012, 2161-2168; Gottlieb et al. 2010, 352-360).

Unfortunately, this therapy is burdened by a reduced compliance by patients that leading them to alternative therapies such as the positional therapy, surgical, orthodontic, that are able to give results superimposable to CPAP and stable in time in a well selected patient groups. The discovery of clinical phenotypes including genotypes with different pathogenic mechanisms has launched a new genetic and biomedical research field that will lead in the future to

new diagnostic and therapeutic strategies increasingly individualized (Malhotra, Orr et Owens 2015, 397-403)

An unmet need in literature is the lack of adherence / compliance to CPAP treatment. It should be evaluated, therefore, the possibility to develop technical / cultural tools in the form of educational / informational interventions able to improve adherence to treatment through an understanding of OSA and its complications. Several studies (Meurice et al. 2007, 37-42) have been conducted to assess the effect of educational / cultural intensive training in OSA and CPAP fields. These studies aim to obtain better results on the adherence of the patient. Interventions that have been conducted include the verbal-visual instruction by health professionals, the applications of the nasal and oral-nasal masks as well as the importance of the disease and its health effects (Jean Wiese et al. 2005, 171-174; Golay et al. 2006, 220-227) with standardized audiovisual presentations and practical demonstrations on performing standards treatments at home. Up to now, there is any "gold standard" training programs in literature that have steadily improved the adherence to CPAP treatment. Many of these clinical trials (Wickwire et al. 2013, 680-693) with double arm (control and study) have given controversial results. These latter studies were also criticized due to the higher level of education of the control arm respect to the study arm, compared to a normally routine care. Consequently, results on the adherence in the study arm has appeared worse. However, the majority of the experts still recommend to all patients that start CPAP, a high level of intensive instruction.

Health-care providers and CPAP: cost-effectiveness of models of care

The Health Technology Assessment is a multidimensional and multidisciplinary approach for the analysis of clinical, social, organizational, economic and legal implications in Italy. This approach undergoes a multi-dimensional evaluation such as the efficacy, safety, cost, and the social and organizational impact (Velardi et al. 2011).

Telemedicine interventions (IT) is a remote communication system of IT / medical data that is used to save time and reduce all the costs spent for managing a home care service for chronic diseases (Toma et al. 2014, 200-211; Park et al. 2014, 65-72). Not surprisingly, a number of clinical studies have been conducted to evaluate the effectiveness of telemedicine interventions (IT) on adherence to CPAP treatment. An expert IT technician has, in fact, produced some interesting data on health procedures on CPAP (Fox et al. 2012, 477-481).

The IT reports were transmitted and received from the individual CPAP home units onto the reference Provider center wirelessly therefore data from the study were collected and processed. Recorded data were represented by: a) loss of pressure in the mask, b) residual AHI c) number of hours of use (5 hours per night). Errors in performing the treatment were easily detected from the technician, who was able to call the patients the next morning through the central Provider and solve any problems about the low efficiency of the treatment. Result were seen after three months: the group assigned to the intervention in telemedicine had 1.88 hours / night of CPAP more compared to the control group with a lower residual AHI.

Another Spanish clinical multicenter randomized controlled trial (Isetta et al. 2015, 1054-1061) used telemedicine for studying economic and clinical impacts as well the improvement of the quality of life (QoL) with CPAP treatment compared to traditional follow-up with face-to-face doctor-patient controls. 139 patients were enrolled with sufficient knowledge of IT world. The follow-up included 3 controls at 1, 3 and 6 months to assess the quality of sleep, the side effects of treatment with CPAP and QoL. In this study were also detected the costs of the treatment and management from the beginning to the end of the study.

Results of the study were that a strategy based on telemedicine for follow-up of CPAP treatment in patients with severe OSAS, was as effective as a therapy performed in hospital in accordance with the gold standard, in terms of the compliance with CPAP and the improvement of the symptoms, with comparable side effects and satisfaction rates. The strategy based on telemedicine had a reduction of total costs in the transport service and productivity.

These new IT telemonitoring systems permit to save operating costs and manage several pa-

tients simultaneously (at least a hundred) using a single provider. However, they hint at possible medico-legal disputes. We try to address them. The first aspect to be defined is the fact that there is no standard of care for telemedicine at international level. Standards of care exist just for services for the individual but there are still not any e-Health practices. Medico-legal issues are: a) respect for personal privacy, b) inaccuracies of self-reporting of patients in data recording, c) the resolution limits of data to be recorded and the consequent delays due to failure / delayed treatment after the recording of data, d) failure of the systems that do not work correctly. In the US, there is a national society in telemedicine named TelaDoc (Gallegos 2015) which features a American National Committee to guarantee certification of electronic systems used in telemedicine, along with the production of evidence-based clinical practice guideline of registered data quality.

Another English multicenter randomized controlled study (McMillan et al. 2015, 1-188) assessed the clinical and economic aspects of the CPAP treatment in OSA elderly patients (PREDICT study). The study have showed that CPAP treatment in these patients (> 65 years) reduced the subjective and objective sleepiness similarly compared to younger patients. Secondary goals were determine the CPAP clinical efficacy, the cost-effectiveness ratio and the real usefulness of the treatment (model-based cost-effectiveness analysis) compared to alternative treatments with APAP / Bilevel / C FLEX (BSC).The QoL at 12 months of treatment was measured by the European Quality of Life-5 Dimensions (EQ-5D);

In elderly patients with OSA, CPAP treatment reduced the somnolence more significantly compared to treatment with APAP / Bi-Level / C FLEX (BSC) over a period of 12 months, improving the EQ-5D.In this recent clinical study (Tan et al. 2015, 525-535) approximately 50% of patients with OSA were intolerant to CPAP, this resulted in the selection of other therapeutic modalities such as surgical treatment. In order to evaluate the cost-effectiveness ratio in intolerant patients, this research group have compared three treatment strategies: (a) no treatment, (b) only CPAP and (c) CPAP followed by surgery (reconstructive palate-pharyngeal surgery (PPRS) or multilevel surgery (MLS]). Obtained results showed that CPAP therapy followed by PPRS (CPAP-PPRS) was the most profitable treatment compared to treatment with single CPAP and compared to the other strategies.

Another interesting prospective randomized controlled trial, published in 2010 by an Italian research group (Vicini et al. 2010, 14-20), have compared the efficacy of the ORL surgery, the maxilla-mandibular advancement (MMA) versus positive ventilation (auto-titrating) APAP. In conclusion, the study has shown that MMA can be a valid alternative therapeutic tool, not lower than the treatment with APAP.

Conclusions

In conclusion, there are many clinical studies that compared the efficacy between the diagnostic / ambulatory treatment versus diagnostic / hospital treatment. All these reports have elucidated that the management strategies for OSA are not clinically inferior to the hospital treatment and can produce similar results compared to the diagnostic / laboratory sleep treatment in hospital (Chai-Coetzer, Antic et McEvoy 2013, 605-615). However, we must point out some important limitations about HST when used in a long-term management strategy, because it can produce: **a**) limited capacity to diagnose sleep disorders other than OSA or non OSA syndrome related to metabolic disorders, neurological; **b**) the need to review / reevaluate the raw data that come automatically without performing a manual analysis of the nocturnal polygraphic tracings; **c**) uncertainties about the long-term use of this outpatient strategy on regards to the overall cost-effectiveness results compared to the hospital diagnostic plan that is based on the supervised polysomnography at 1st level.

There are also new emerging results in the literature that support the role of non-medical health professionals expert in sleep study such as nurses, care giver, IT / health care, who are able to manage the home care OSA in a cost-effective way. We still need of more long-term prospective studies that can evaluate the cost-effectiveness ratio, including direct and indirect costs of hospital management models versus models that take into account new qualified

non-medical personnel care. Finally, future knowledge in this area will come from the application of new technologies. Olivia J.et al (Walch, Cochran et Forger 2016, e1501705) to have shown that the sleep wake rhythm is changed from a social plan. They used smartphone app that have developed, ENTRAIN, accurately collects data on sleep habits around the world. This work better defines and personalizes "normal" sleep, produces hypotheses for future testing in the laboratory, and suggests important ways to counteract the global sleep crisis Moreover, we use mobile technology to collect a massive data set at essentially no cost. In analyzing these large data sets, mathematical modeling will be key to generating useful predictions from the unstructured bulk collection

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Uterine rupture: up to date

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Abstract
The uterine rupture is a dramatic obstetrical event, fortunately unusual. Unfortunately, onset is often sudden, very often during pregnancy, in the third trimester, and during labor. The uterine rupture depends on a number of biological and mechanical factors, in which the muscular scarring process is the main responsible factor. The biology of uterine muscle scarring depends on a set of biomolecular processes involving growth factors, neurotransmitters, neoangiogenesis, deposition and reabsorption of collagen fibers, ect. Any interference in this precess creates the conditions for the uterine rupture, which occurs under contract, during the maximum muscular tension. Separation of the fibers almost always involves a traumatic vascular interruption with copious haemorrhage. Here is why uterine rupture is a very rare event, but very dangerous for the life of the woman and the fetus inside it.

Keywords: Uterine rupture, pregnancy, myomectomy, fibroids, neonagiogenesis, growth factors

Introduction

Uterine rupture is an uncommon but life-threatening obstetric complication. It leads to high maternal and perinatal morbidity and mortality, and it is complete or incomplete. It may be incomplete, when uterine serosa remains intact, or complete, in cases of disruption of the full-thickness of uterine wall including uterine serosa, thus resulting in a direct connection between the peritoneal space and the uterine cavity with or without protrusion or expulsion of the fetus and/or placenta into the peritoneal cavity. The uterine rupture may be spontaneous, traumatic or the result of the scar dehiscence (Guseh et al. 2016, 255-67).
It can either occur in women with (Gardeil, Daly et Turner 1994, 107-10) a native, unscarred uterus or a uterus with a surgical scar from previous surgery.
It can occur during pregnancy, early in labor or following the prolonged labor, most frequently near or at term. Rarely, uterus can rupture during early to mid-pregnancy (Guseh et al. 2016, 255-67).
Any surgical insult to the uterus can lead to uterine dehiscence and rupture. Nevertheless, different surgical procedures and techniques may cause a different healing process, thus causing differences in the uterine rupture rate. Anyway, the initial symptoms and signs of uterine rupture are typically nonspecific, which makes the diagnosis difficult and sometimes delays definitive therapy. From the time of diagnosis to delivery, generally only 10-37 minutes are available before clinically significant fetal morbidity becomes inevitable. Fetal morbidity occurs as a result of catastrophic hemorrhage, fetal anoxia, or both (Nahrum et Pham).

Incidence

From 1976-2012, 25 peer-reviewed publications described the incidence of uterine rupture, and these reported 2,084 cases among 2,951,297 pregnant women, yielding an overall uterine rupture rate of 1 in 1,146 pregnancies (0.07%) (Nahrum et Pham).

The overall incidence of uterine rupture published from less developed countries is 9.3% of deliveries; in fact in developing countries this rate is much higher than in the developed countries for obstructed labor (Rajaram, Agrawal et Swain 2017, 7-10).

In the Second Report on Confidential Enquiries into Maternal Deaths in South Africa 1999–2001, ruptured uterus caused 6.2% of deaths due to direct causes and 3.7% of all deaths (1.9% due to rupture of an unscarred uterus and 1.8% due to rupture of a scarred uterus) (Gülmezoglu et al. 2004, 16).

No estimates exist to assess the magnitude of this potentially life-threatening condition.

Ofir et al found that the risk for uterine rupture among women who had not undergone previous Cesarean section (CS) is 0.02%, and overall risk of uterine rupture is 0.035% of all singleton deliveries (Ofir et al. 2004, 425-29).

The reported incidence of the uterine rupture of an unscarred uterus in developed world is near 1:10000-20000 deliveries (Parant 2012, 803-16). In developing countries, the published incidence of uterine rupture varies from 1,45 to 25%, with 25% in Ethiopian woman with obstructed labor (Berhe et Wall 2014, 695-707).

A 10-year Irish study by Gardeil et al showed that the overall rate of unscarred uterine rupture during pregnancy was 1 per 30,764 deliveries (0.0033%). No cases of uterine rupture occurred among 21,998 primigravidas, and only 2 (0.0051%) occurred among 39,529 multigravidas with no uterine scar (Guseh et al. 2016, 255-67).

A meta-analysis of 8 large, modern (1975-2009) studies from industrialized countries revealed 174 uterine ruptures among 1,467,534 deliveries. This finding suggests that the modern rate of unscarred uterine rupture during pregnancy is 0.012% (1 in 8,434). This rate of spontaneous uterine rupture has not changed appreciably over the last 50 years, and most of these events occur at term and during labor. An 8-fold increased incidence of uterine rupture of 0.11% (1 in 920) has been noted in developing countries, with this increased incidence of uterine rupture having been attributed to a higher-than-average incidence of neglected and obstructed labor due to inadequate access to medical care (Nahrum et Pham).

Schrinsky and Benson reported 22 cases of uterine rupture in gravidas with unscarred uteri. Nineteen occurred during labor (86%), and 3 occurred before labor (14%). This percentage was markedly different from that of gravidas with a previous uterine scar, for whom the timing of uterine rupture between labor and the antepartum period was nearly evenly distributed (Schrinsky et Benson 1978, 217-32).

Author conduced a review to evaluate the overall incidence of rupture of a uterus with a previous CS scar for World Health Organization (WHO). For unselected pregnant women, the prevalence of uterine rupture reported was considerably lower for community-based (median 0.053, range 0.016–0.30%) than for facility-based studies (0.31, 0.012–2.9%). The prevalence tended to be lower for countries defined by the United Nations as developed than the less or least developed countries. For women with previous caesarean section, the prevalence of uterine rupture reported was in the region of 1%. Only one report gave a prevalence for women without previous caesarean section, from a developed country, and this was extremely low (0.006%) (Gülmezoglu et al. 2004, 16).

Currently, the problem of uterine rupture is related, for developing countries, to the increasing rate of cesarean delivery. The increasing number of woman presenting with scared uterus either from Cesarean Section (CS) or uterine surgery leads to an increase in the number of women exposed to the uterine rupture risk.

A Norwegian study published in the Journal has found that for women with previous CS, the risk of uterine rupture was 8 times higher after trial of labour (TOL) than at repeated elective CS. Induction of labor (using prostaglandins) was associated with the highest risk of uterine rupture (Al-Zirqi et al. 2010, 809-20).

The American college of Obstetricians and Gynecologists (ACOG) estimated the risk of uterine rupture in women with a previous CS and

concluded that the lower segment caesarean scar has a minimum risk (0.2-1.5%) of rupture during vaginal delivery (AA. VV. 1999, 201-8).
Canadian study, trial of labor following previous CS was associated with increased risk of uterine rupture (by 0.56%), but fewer maternal deaths than elective CS (1.6 vs 5.6 per 100,000) (Wen et al. 2004, 1263-69).
A trial of labor following a previous CS increases the risk of uterine rupture compared to the elective repeat cesarean section. The risk is also influenced by the number of previous cesarean deliveries and on whether the labor is induced, augmented or spontaneous. The inter-delivery interval may also influence this risk.
In a study by Lydon-Rochelle et al of 10 789 patients with a single previous CS, who later labored spontaneously in singleton pregnancy, the uterine rupture rate was 0.52% (Lydon-Rochelle et Cahill 2010, 249-57).
In cases of trial of labor in women with previous low vertical scar uterine rupture rate is 1.1% and the trial of labor following previous CS with unknown type of cesarean scar is associated with the rate of 0.56% of uterine rupture (Nahrum et Pham).

Risk factors for uterine rupture

The major characteristics for determining the risk of uterine rupture in pregnant are listed below.
Uterine status is either unscarred or scarred. Scarred status may include previous cesarean delivery, including the following: single low transverse (further subcategorized by 1-layer or 2-layer hysterectomy closure); single low vertical; classic vertical; multiple previous cesarean deliveries (Nahrum et Pham).
Scarred status may also include previous laparoscopic or laparotomic myomectomy (Hagneré et al. 2011, 162-65).
In case of uterine rupture, uterine morphology may be regular or may involve a congenital uterine anomaly (Shahid et al. 2010, 121-25). Pregnancy considerations include the following traditional risk factors: grand multiparity, maternal age, placentation (accreta, percreta, increta, previa, abruption), cornual (or angular) pregnancy, uterine overdistension (multiple gestation, polyhydramnios), dystocia, fetal macrosmia, contracted pelvis, gestation longer than 40 weeks, trophoblastic invasion of the myometrium (Ofir et al. 2003, 1042-46).
The risk factors for labor are as follows: patient not in labor, patient with spontaneous labor, induced labor - with oxytocin, with prostaglandins, augmentation of labor with oxytocin, duration of labor and obstructed labor (Goyal 2009, 1117-23).
Obstetric management risk factors for uterine rupture include the following: operative delivery (forceps or vacuum); intrauterine manipulation (external cephalic version, internal podalic version, breech extraction, shoulder dystocia, manual extraction of placenta); fundal pressure (i.e. Kristeller maneuver) (LANG et LANDON 2010, 237-51).
Uterine trauma linked to uterine ruptures are the following: direct uterine trauma (eg, motor vehicle accident, fall), violence (eg, gunshot wound, blunt blow to abdomen), rupture of the unscarred uterus (Uccella et al. 2011).
The normal, unscarred uterus is least susceptible to rupture. Grand multiparity, neglected labor, malpresentation, breech extraction, and uterine instrumentation are all predisposing factors for uterine rupture (Guseh et al. 2016, 255-67).

The problem of uterine scar

Women with prior myomectomy or prior traditional CS often have an early cesarean delivery because of concern for uterine rupture. The problem is related to the quality of the uterine scar. Unfortunately, until now, nobody has been able to perform the "in vivo studies" on the uterine muscle, the myometrium, as it is not easy to make a monitoring of a scar tissue which the myometrium, appropriately, all studies performed are indirectly, by growth factors and proteins, by imaging, or by other non-invasive methods. All studies conducted so far have the limitation of poor biological reliability. The problem is not of simple solution in that the uterine scar is the weak of the myometrium, which, subjected to the contraction stress, can suddenly breaking down. Unfortunately, we must be content of reports and investigations on overall and selected rates of uterine ruptures. In fact, the rate of uterine rupture recently published in developed countries following

classical cesarean delivery is 0.88 %, which is much lower than the quoted rate of up to 9% in women with prior classical cesarean who labor (Gülmezoglu et al. 2004, 16).
This is probably due to elective late preterm delivery in such cases. The frequency of uterine rupture following prior classical cesarean delivery in labor is 1.8% (Gyamfi-Bannerman et al. 2012, 1332-37).
The uterine rupture rate in women with previous classic, inverted T or J incision who either refused repeat cesarean delivery or presented in labor in Landon et al study was 1.9% (Landon et Lynch 2011, 257-61).

Uterine rupture after myomectomy

The same trend is followed for delivery for women with prior myomectomy because it is thought that the scar from myomectomy is functionally equivalent to the scar from classical cesarean delivery (Qahtani 2013, 214-19).
One of the first author who investigated uterine ruptures after myomectomy was, in 1964, Garnet who identified 3 (4%) uterine ruptures among 83 women who had scars from a previous abdominal myomectomy (Garnet 1964, 898-905).
The prevalence of uterine rupture following myomectomy - all types of surgery – is 0.79 % and it is comparable with that after cesarean section. Based on the available evidence, there is no significant difference between the incidences of a rupture during pregnancy following a laparoscopic (1.2 %) versus an open myomectomy (0.4 %) (Claeys et al. 2014, 197-206).
It is not clear whether the laparoscopic procedure is associated with higher risk of subsequent rupture or, whether, these cases are being more systematically reported. It is also clear that the location and size of the fibroids might affect the likelihood of uterine rupture following previous myomectomy, and the difference may be partially explained by confounding factors. Between 1970 and 2013, there has been an overall increase in the CS rates leading to a higher primary C-section rate in the last two decades (the "era of laparoscopy") compared to the era of open surgery. Indeed, in the last two decades, the laparoscopic approach has become the preferred technique, with laparoscopic suturing requiring more than average technical expertise. Furthermore, it is striking to observe that a uterine rupture in a woman following a myomectomy almost exclusively occurs during pregnancy and very exceptionally during active labor, as opposed to following a prior cesarean section. This can be explained to differences in the site of the incision with the majority of myomectomies being done in the corporeal part of the womb as opposed to the lower uterine segment in the case of cesarean delivery. Thus, Claeys et al concluded that the risk of a uterine rupture following a myomectomy regardless of the technique used seems very rare (less than 1 % of the ongoing pregnancies) (Claeys et al. 2014, 197-206).
Literature data suggest that the overall uterine rupture rate following myomectomy is 0.2% (Claeys et al. 2014, 197-206).
A true evaluation of the uterine rupture rate after endoscopic myomectomy is difficult, as information about this comes, primarily, from case reports (Pistofidis et al. 2012).
Anyway, uterine rupture following myomectomy is one of the major complications of myomectomy. In the light of advanced age of obstetric population, there is a potential risk of uterine rupture on the site of previous myomectomy scar. Both myomectomy and CS can be, either directly or indirectly, predisposing factors of abnormally invasive placenta, influencing the risk of uterine rupture. The influence of myomectomy technique on the incidence of the rupture is still a matter of debate, even if Tinelli et all published studies showing the reduced incidence of uterine rupture by intracapsular myomectomy myometrial sparing (Tinelli et al. 2016, 129-39).
The myometrial healing following myomectomy is affected by the method and/or instrumentation used during surgery. Uterine incision, achievement of hemostasis and closing the myometrial defect, the extent of tissue damage (influenced by myoma characteristics such as type, size and number), the potential formation of hematoma within the myometrium, gas pneumoperitoneum in laparoscopic procedures, and patients individual characteristics influence the healing process (Mynbaev et al. 2016, 1013-15; Tinelli et al. 2012, 119-29).

It is more difficult to make an adequate suture by laparoscopy than by laparotomy. At laparotomy, closure of the myometrial defect is usually accomplished by a multilayered suture. During laparoscopy, failure to suture adequately myometrial defects, lack of hemostasis with subsequent hematoma formation may interfere with wound healing and increase the risk of rupture (Mettler et al. 2012, 1-8).

Inappropriate use of electrocautery may induce in-depth necrosis of the myometrium with an adverse effect on healing. Excessive use of diathermocoagulation (with inflammation, necrosis, fibrosis, neuropeptides damaging) can lead to delay in the correct uterine healing and generate a weaker uterine scar (Tinelli et Malvasi 2015, 73-93).

In a review, one rupture occurred on the site of later myomectomy in another institute, due to placenta percreta over the second scar. Although the authors did not calculate this case in their count, second myomectomy was the most probable causative mechanism of forming an abnormally invasive placenta. The other rupture case had a rupture on the site of myomectomy scar which was re-sutured during second-look laparoscopy 7 weeks after the surgery (Parker et al. 2010, 551-54).

Pistofidis and coworkers investigated all 7 cases of uterine rupture after laparoscopic myomectomy reported to the Greek Board of Endoscopisc Gynecologic Surgery from 1998 to 2011. Only one of those patients had intramural myoma, and the endometrial cavity was not opened in any of the patients. Bipolar diathermy was the sole method of hemostasis in 28.6% of cases, and could be characterized as excessive in 87.5% of patients. Most of the ruptures occurred at 34 weeks of gestation or later, with 1 case at 24 weeks of gestation in twin pregnancy. Those authors concluded that it seems reasonable that women who have undergone laparoscopic myomectomy would best avoid multiple pregnancies because of potentially increased risk of rupture (Pistofidis et al. 2012).

Parker et al. investigated 19 cases of uterine rupture following laparoscopic myomecyomy and concluded that its reasonable to use in laparoscopy to techniques similar to those adoptes for open myomectomy as bipolar diathermy during laparoscopic procedures has potentially detrimental effect on the healing process (Parker et al. 2010, 551-54).

Sizzi et al. in a multicentric study of laparoscopic myomectomy complications reported 1 rupture among 386 pregnancies (0.26%) out of 2050 operations (Sizzi et al. 2007, 453-62).

Robotic-assisted laparoscopic surgery is relatively new innovation in the field of gynecologic surgery. An advantage of robotic-assisted laparoscopic myomectomy is the ability to perform an identical multilayer closure to the abdominal approach that controls hemostasis without the need for significant use of electrosurgical instruments (Rossi et Prefumo 2015, 273-80; Tinelli et al. 2011, 12-24).

The incidence of uterine rupture in pregnancy after robotic-assisted myomectomy reported by Pitter et al is 1.1%, which is incomparable with the previously reported incidence after conventional laparoscopic myomectomy (Claeys et al. 2014, 197-206). The uterine rupture occurred, in this study, in a patient at 33 weeks of gestation; such patient conceived 18 weeks after the robotic multiple myomectomy without entering the endometrial cavity.

The real recurrent uterine rupture rate, in patients with prior repair is unknown. In the Pistofidis study, out of 7 cases of uterine rupture after laparoscopic myomectomy there were two cases of recurrent rupture (28.6%) (Pistofidis et al. 2012).

The incidence of peripartum hysterectomy following uterine rupture recently reported by Charach and Sheiner is the 20.7% (Charach et Sheiner 2013, 1196-1200). The independent risk factors for peripartum hysterectomy following uterine rupture are: relaparotomy, extended tear involving uterine cervix, severe bleeding requiring packed cells transfusion and grand multiparity (Charach et Sheiner 2013, 1196-1200).

Although CS and repeated CS were found to be separate risk factors for uterine rupture and emergency peripartum hysterectomy in previous publications, cited authors documented a significantly reduced number of hysterectomies following uterine rupture in the women who underwent CS or had a previous CS, thus expressing the importance of fertility preserving surgery in modern obstetrics and use of hyster-

ectomy as last option procedure (Qahtani 2013, 214-19).
It is more commonly necessary in cases of traumatic or spontaneous rupture, and its incidence in such cases in some reports has been 85% (Charach et Sheiner 2013, 1196-1200).

Spontaneous uterine rupture

Traditionally, primigravidae and women with unscarred uterus are considered immune to rupture. Spontaneuos uterine rupture usually occurs in labor. Rupture of an unscarred uterus is a rare event, as the majority of uterine rupture during pregnancy involves scarred uterus. Rupture of an unscarred uterus is a rare event involving 1 : 5,700–20,000 deliveries (Ofir et al. 2004, 425-29).
This frequency is often higher in developing countries, where it can reach 75% of cases in some areas (Guèye et al. 2012, 598356).
In a study of uterine ruptures in The Netherlands, the incidence of rupture in unscarred and scarred uteri was 0.7 and 5.1 per 10,000 deliveries, respectively; ruptures of unscarred uteri accounted for 13 percent of all ruptures (Al-Zirqi et al. 2016, 780-87).
Although uncommon in nuliparous woman, spontaneous uterine rupture is described in juveniles black African women due to contracted pelvis [5,7].
A major factor in spontaneous uterine rupture is obstructed labor, especially in the developing world (Berhe et Wall 2014, 695-707).
Schrinsky and Benson reported 22 cases of uterine rupture in gravidas with unscarred uteri. Nineteen occurred during labor (86%), and 3 occurred before labor (14%). This percentage was markedly different from that of gravidas with a previous uterine scar, for whom the timing of uterine rupture between labor and the antepartum period was nearly evenly distributed (Schrinsky et Benson 1978, 217-32).
Rupture of an unscarred uterus may be caused by trauma or congenital or acquired weakness of the myometrium, such as collagen disease (McCarthy et Germain 2013, 71-80). Sources of trauma include motor vehicle accidents and obstetric maneuvers (eg, internal or external version).
Clinical signs of uterine rupture during pregnancy are nonspecific and can be confusing. Indeed, it is not always easy to distinguish it with other abdominal emergencies (appendicitis, gallstones, pancreatitis, etc.) (Suner et al. 1996, 181-85).
Early surgical intervention is usually the key to successful treatment of uterine rupture. The therapeutic management is a total or subtotal hysterectomy. The suture can be performed and helps to preserve the reproductive function of patients who have never given birth with a recurrence risk of uterine rupture assessed between 4 and 19% at a subsequent pregnancy. For this reason, it has been recommended that women with a previous uterine rupture undergo an elective Caesarean delivery as soon as fetal lung maturity can be demonstrated (Guèye et al. 2012, 598356).

Uterine rupture after cesarean section

The effect of previous cesarean delivery on the risk of uterine rupture has been studied extensively. In a meta-analysis, Mozurkewich and Hutton used pooled data from 11 studies and showed that the uterine rupture rate for women undergoing a trial of labor after cesarean section (TOLAC) was 0.39% compared with 0.16% for patients undergoing elective repeat cesarean delivery (odds ratio [OR], 2.10; 95% CI, 1.45-3.05). After restricting the meta-analysis to 5 prospective cohort trials, similar results were found (OR, 2.06; 95% CI, 1.40-3.04) (Mozurkewich et Hutton 2000, 1187-97).
Hibbard et al examined the risk of uterine rupture in 1,324 women who underwent a TOLAC. They reported a significant difference in the risk of uterine rupture between women who achieved successful vaginal birth compared with women in whom attempted vaginal delivery failed (0.22% vs 1.9%; OR, 8.9; 95% CI, 1.9-42) (Hibbard et al. 2001, 1365-73).
The effect of previous CS on the rate of subsequent pregnancy-related uterine rupture have been evaluated by investigations on vaginal birth after cesarean section (VBAC).
The overall rate of VBAC in the United States increased from 3.4% in 1980 to a peak of 28% in 1996. Commensurate with this 8-fold in-

crease in the VBAC rate, reports of maternal and perinatal morbidity also increased, in particular with reference to uterine rupture. By 2007, the VBAC rate in the United States had fallen nationally to 8.5%. Not surprisingly, the cesarean delivery rate also reached an all-time high of 32% in 2007. In its most recent guidelines pertaining to VBAC in August 2010, the American Congress of Obstetricians and Gynecologists (ACOG) adopted the recommendation not to restrict women's access to VBAC (AA. VV. 2010, 450-63).

About traditional CS by vertical incision, in a meta-analysis, Rosen et al reported an 11.5% absolute risk of uterine rupture (3 of 26 cases) in women with classic vertical cesarean scars who underwent an unplanned TOLAC (Rosen, Dickinson et Westhoff 1991, 465-70).

For women who underwent repeat cesarean section, Chauhan et al reported that the uterine rupture rate for 157 women with prior classical uterine cesarean scars was 0.64% (95% CI, 0.1-3.5%). All patients in that study underwent repeat cesarean delivery, but a high rate of preterm labor resulted in 49% of the patients being in labor at the time of their cesarean delivery (Chauhan et al. 2002, 946-50). Chauhan et al observed also a 9% rate of asymptomatic uterine scar dehiscence (95% CI, 5-15%).

Landon et al (Landon et al. 2004, 2581-89) reported a 1.9% absolute uterine rupture rate (2 of 105 cases) in women with a previous classic, inverted *T,* or *J* incision who either presented in advanced labor or refused repeat cesarean delivery. This meta-analysis of pooled data from 5 studies demonstrated a 1.1% absolute risk (12 of 1,112 cases) of symptomatic uterine rupture in women undergoing a TOLAC with a low vertical cesarean scar (Landon et al. 2004, 2581-89). Compared to women with low transverse cesarean scars, these data suggest no significantly increased risk of uterine rupture or adverse maternal and perinatal outcomes.

For 322 pregnancies that occurred after a low vertical cesarean delivery, the overall rate of uterine rupture was 0.62%. This rate could be further divided as 1.15% for 174 women who underwent a TOLAC compared with no ruptures among 148 women who underwent elective repeat cesarean delivery (Naef et al. 1995, 1666-74).

The Maternal-Fetal Medicine Units (MFMU) Network cesarean delivery registry reports a 0.5% risk (15 of 3,206) of uterine rupture for patients who underwent a TOLAC with an unknown uterine scar (Landon et al. 2004, 2581-89).

For cases in which there are 1 or 2 unknown prior uterine incisions, there is a single small, randomized, controlled trial by Grubb et al that compared labor augmentation with oxytocin (n=95) with no intervention (n=93) in women with prior cesarean deliveries involving either 1 or 2 unknown uterine incisions. Four uterine dehiscences and 1 uterine rupture occurred, all in the group that underwent labor augmentation. In the 1 case of uterine rupture, the unknown uterine scar was in a patient with 2 prior cesarean deliveries, one of which involved a vertical incision. Had the uterine scar status for this patient been known in advance, it would have represented a contraindication to TOLAC (Naef et al. 1995, 1666-74).

In a study of 20,095 women by Lydon-Rochelle et al (Lydon-Rochelle et Cahill 2010, 249-57), the spontaneous uterine rupture rate among 6,980 women with a single cesarean delivery scar who underwent scheduled repeat cesarean delivery without a TOL was 0.16%. This investigation showed that the uterine rupture rate among 10,789 women with a single previous cesarean delivery who labored spontaneously during a subsequent singleton pregnancy was 0.52% (Lydon-Rochelle et Cahill 2010, 249-57). This rate of uterine rupture implies an increased relative risk (RR) of 3.3 (95% CI, 1.8-6.0) for women who labor spontaneously compared with women who undergo elective repeat cesarean delivery.

This finding indicates that uteri with cesarean scars have an intrinsic propensity for rupture that exceeds that of the unscarred organ during pregnancy, which is 0.012% (OR increase of approximately 12-fold). Therefore, all other uterine rupture rates in women with a previous cesarean delivery should be referenced to this expected baseline rate.

In a study by Ravasia et al of 1,544 patients with a previous cesarean delivery who later labored spontaneously, the uterine rupture rate was 0.45% (Ravasia, Wood et Pollard 2000, 1176-79).

Zelop et al found that, among 2,214 women with 1 previous cesarean delivery who labored spontaneously, the uterine rupture rate was 0.72%. The authors of this article performed a meta-analysis of 29,263 pregnancies from 9 studies from 1987-2004 and showed that the overall risk of uterine rupture was 0.44% for women who labor spontaneously after a previous cesarean delivery (Ravasia, Wood et Pollard 2000, 1176-79).

Uterine rupture in previous CS, with oxytocin and induction of labor

The use of oxytocin during labor in patients with previous CS is a very little used practice for related fear of uterine rupture. Thus, very few studies have stratified their data by labor augmentation versus labor induction and the data that do exist are conflicting. There is wide variance in the frequency of clinical use of oxytocin as well as in the dose and dosing schedules of oxytocin that are used. It is therefore not possible to draw complete conclusions about the related risk of uterine rupture in such patients.

On the contrary, current ACOG guidelines discourage the use of prostaglandins to induce labor in most women with a previous cesarean delivery. This recommendation is based on considerable evidence for an increased risk of uterine rupture associated with prostaglandins.

Blanchette et al (Blanchette et al. 2001, 1478-87) reported the rate of uterine rupture for 288 women who underwent oxytocin augmentation of labor after a previous cesarean delivery; it was 1.4%, compared with 0.34% for 292 women who underwent a trial of spontaneous labor. This finding suggests a 4-fold increased risk of uterine rupture in women who undergo labor augmentation with oxytocin compared with spontaneous labor after a previous cesarean delivery.

In the MFMU Network study, the rate of uterine rupture with oxytocin augmentation was 0.9% (52 of 6,009 cases) versus 0.4% (24 of 6,685 cases) without oxytocin use. In contrast, a meta-analysis of studies published prior to 1989 found that the use of oxytocin was unassociated with uterine rupture (National Institutes of Health 2010, 351-65).

Zelop et al also found that labor augmentation with oxytocin did not significantly increase the risk for uterine rupture (Ravasia, Wood et Pollard 2000, 1176-79).

However, conclusions of such studies are both limited and suspect because, in general, no proper adjustment has been made for the potential (and very likely) confounding-by-indication that occurs in the observational studies that attempt to compare the rate of uterine rupture for women receiving treatment with oxytocin versus those who do not.

On the contrary, emerging data indicate that induction of labor after a prior cesarean delivery appears to be associated with an increased risk of uterine rupture.

Zelop et al found that the rate of uterine rupture in 560 women who underwent labor induction after a single previous cesarean delivery was 2.3% compared with 0.72% for 2,214 women who had labored spontaneously (P =.001) (Ravasia, Wood et Pollard 2000, 1176-79).

In a study by Ravasia et al of 575 patients who underwent labor induction, the uterine rupture rate was 1.4% compared with 0.45% for women who labored spontaneously (P =.004) (Ravasia, Wood et Pollard 2000, 1176-79).

Blanchette et al (Blanchette et al. 2001, 1478-87) found that the uterine rupture rate after previous cesarean delivery when labor was induced was 4% compared with 0.34% for women who labored spontaneously. This last finding suggests a 12-fold increased risk of uterine rupture for women who undergo labor induction after previous cesarean delivery.

Bujold et al found no statistically significant difference among the uterine rupture rates of 1.1% for spontaneous labor, 1.2% for induction by amniotomy with or without oxytocin, and 1.6% for induction by transcervical Foley catheter (P =0.81) (Bujold, Blackwell et Gauthier 2004, 18-23).

Hoffman et al reported a 3.67-fold increased risk of uterine rupture (95% CI, 1.46-9.23) with Foley catheter use for preinduction cervical ripening. Importantly, however, many of these patients received concomitant oxytocin together with application of the transcervical Foley catheter (Hoffman et al. 2004, 217-22).

Pettker et al (Pettker et al. 2008, 1320-26) found that the addition of oxytocin to the use of a transcervical Foley catheter for labor induction does not shorten the time to delivery and has no effect on either the likelihood of delivery within 24 hours or the vaginal delivery rate.

In a systematic review that evaluated maternal and neonatal outcomes following induction of labor (4,038 women) and spontaneous labor (13,374 women) in women who previously underwent cesarean section, Rossi & Prefumo reported a lower incidence of vaginal delivery with induced labor but higher rates of uterine rupture/dehiscence, repeat cesarean section, and postpartum hemorrhage (Rossi et Prefumo 2015, 273-80).

Facchinetti et al (Facchinetti et al. 2015, 55-58) indicated that women with a previous cesarean delivery being induced for premature rupture of membranes and who have a favorable Bishop have a higher likelihood of success.

Signs, symptoms and diagnosis of uterine rupture

Classical signs and symptoms of the uterine rupture have been reported in 19th century and the signs and symptoms of uterine rupture largely depend on the timing, site, and extent of the uterine defect (Guseh et al. 2016, 255-67). Prolonged, late, or recurrent variable decelerations or fetal bradycardias are often the first and only signs of uterine rupture (Gardeil, Daly et Turner 1994, 107-10).

The most common clinical presentation is sudden appearance of fetal distress during labor and maternal shock. The classic signs and symptoms of uterine rupture are (1) fetal distress (as evidenced most often by abnormalities in fetal heart rate), (2) diminished baseline uterine pressure, (3) loss of uterine contractility, (4) abdominal pain, (5) recession of the presenting fetal part, (6) hemorrhage, and (7) shock. This typical clinical presentation is rarely present, and in some cases, uterine rupture is incidental finding on laparotomy (Guseh et al. 2016, 255-67).

Uterine rupture at the site of a previous uterine scar is typically less violent and less dramatic than a spontaneous or traumatic rupture because of their relatively reduced vascularity (Golan, Sandbank et Rubin 1980, 549-54; Guseh et al. 2016, 255-67; Gardeil, Daly et Turner 1994, 107-10).

Sudden or atypical maternal abdominal pain occurs more rarely than fetal heart rate decelerations or bradycardia. In 9 studies from 1980-2002, abdominal pain occurred in 13-60% of cases of uterine rupture. In a review of 10,967 patients undergoing a TOLAC, only 22% of complete uterine ruptures presented with abdominal pain and 76% presented with signs of fetal distress diagnosed by continuous electronic fetal monitoring (Revicky et al. 2012, 665-73).

In a study of Bujold and Gauthier (Bujold et al. 2002, 1199-1202), abdominal pain was the first sign of rupture in only 5% of patients and occurred in women who developed uterine rupture without epidural analgesia but not in women who received an epidural block.

So, abdominal pain is an unreliable and uncommon sign of uterine rupture. Initial concerns that epidural anesthesia might mask the pain caused by uterine rupture have not been verified and there have been no reports of epidural anesthesia delaying the diagnosis of uterine rupture. The ACOG guideline from 2010 suggests there is no absolute contraindication to epidural anesthesia for a TOLAC because epidurals rarely mask the signs and symptoms of uterine rupture.

The diagnosis of uterine rupture is complex and often relied on the experience and intuition of clinicians. Several reports have suggested that transabdominal, transvaginal, or sonohysterographic ultrasonography may be useful for detecting uterine-scar defects after cesarean delivery. Rozenberg et al prospectively examined 642 women and found that the risk of uterine rupture after previous cesarean delivery was directly related to the thickness of the lower uterine segment, as measured during transabdominal ultrasonography at 36-38 weeks of gestation. The risk of uterine rupture increased significantly when the uterine wall was thinner than 3.5 mm. Using a 3.5 mm cutoff, the authors had a sensitivity of 88%, specificity of 73.2%, positive predictive value of 11.8%, and a negative predictive value of 99.3% in predicting subsequent uterine rupture (Rozenberg et al. 1999, 39-45).

In a study of 722 women, Gotoh et al (Gotoh et al. 2000, 596-600) reported that a uterine wall thinner than 2 mm, as determined with ultrasonography performed within 1 week of delivery, significantly increased the risk of uterine rupture. Positive and negative predictive values were 73.9% and 100%, respectively.

Conclusion

Uterine rupture is a rare but often catastrophic obstetric complication with an overall incidence of approximately 1 in 1,536 pregnancies (0.07%). In developed countries, the uterine rupture rate during pregnancy for a woman with an unscarred and normal uterus is 1 in 8,434 pregnancies (0.012%). Uterine ruptures occur, generally, in scarred uteri, most of which are the result of previous myomectomy or/and CSs. A single cesarean scar increases the overall rupture rate to 0.5%, with the rate for women with two or more cesarean scars increasing to 2%. Other subgroups of women who are at increased risk for uterine rupture are those who have a previous single-layer hysterotomy closure, a short interpregnancy interval after a previous CS, a congenital uterine anomaly, a macrosomic fetus, prostaglandin exposure, and a failed previous trial of a vaginal delivery.
Surgeon has less than 10-37 minutes after uterine rupture to minimize the risk of permanent perinatal injury to the fetus, even if often the damage is not preventable (Nahrum et Pham).
The general clinical early indicator of uterine rupture is the onset of a prolonged, persistent, and profound fetal bradycardia. Other signs and symptoms, such as abdominal pain, abnormal progress in labor, and vaginal bleeding, are less consistent and less valuable than bradycardia in establishing the appropriate diagnosis.
Generally, the obstetricians should be able to start cesarean delivery within 20-30 minutes of a diagnosis of fetal distress is of minimal utility with respect to uterine rupture. In the case of fetal or placental extrusion through the uterine wall, irreversible fetal damage can be expected before that time.

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First evaluation of the ASL of Lecce mammographic screening program results by using Surgical Pathology's indicators of quality in diagnosis and treatment

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Abstract
The aim is to highlight the progress of earliness and quality of diagnosis and breast cancer cure in the Province of Lecce by surveying a number of indicators obtainable from surgical pathologist's evaluation. The study, conducted with the software SQTM (www.qtweb.it), is based on breast cancer of women 50-69 years old residing in the Province of Lecce who undergone breast surgery in the years 2003, 2004, 2010, 2011 and 2012 at the "V. Fazzi " Hospital, which is reference center for diagnosis and treatment of breast cancer within the Breast Cancer Screening Program of the ASL of Lecce , active since 2008. Compared to the prescreening period, results of all the indicators showed an almost progressive and significant improvement along the years, highlighting the first beneficial effects, primarily the improvement of early diagnosis, resulting from the impact of screening on the female target population. However, the study has emphasized some important problems arising from persistent and systematic deficiencies in the organizational and multidisciplinary approach, on which we must concentrate efforts to further improve the screening program results.

Keywords: Breast Cancer, Diagnosis Quality, Mammographic Screening

Introduction

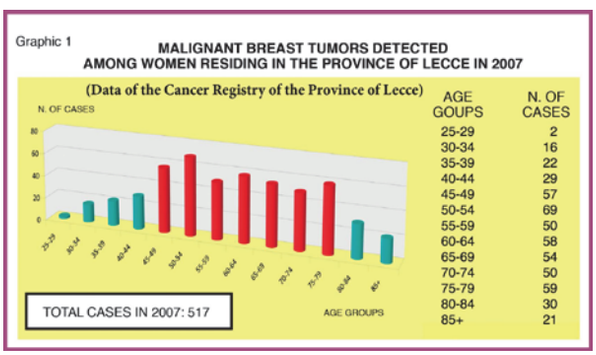
The Planned Mammographic Screening (PMS) for breast cancer, with the uterine cervix cancer screening and the colon-rectum one's, is part of LEA (Essential Assistance Levels) in Italy from several years, implying the national and regional sanitary service assure all the women from the target population (50-69 year old) of active measures for early diagnosis, based on mammography as the first level test carried out after a call once every two years. The planned screening's first aim is to reduce mortality for all the neoplasias is addressed to, with an acceptable cost/benefit ratio. The second aim is to improve the diagnostic and therapeutic course quality. So a planned screening program is not limited to appropriate organizational measures for a good coverage and compliance of the target population in first level testing , it provides also a good management of a right diagnostic and therapeutic course, a control system and checking the results and a continuous action to straighten the shot of screening organizational machine. From 2006 is available the SQTM (computerized Schedule for Quality in Treatment of Mammary carcinoma) dedicated software developed by Piedmont's CPO (regional Oncological Prevention Center) with European funds from the "Europe against cancer" Program and under the auspices of EUSOMA. SQTM is used from several years by GISMa (Mammographic Screening Italian Group) to do year's surveys on mammographic screening results in Italy, based on monitoring

many quality's indicators of diagnosis and treatment.

Objective

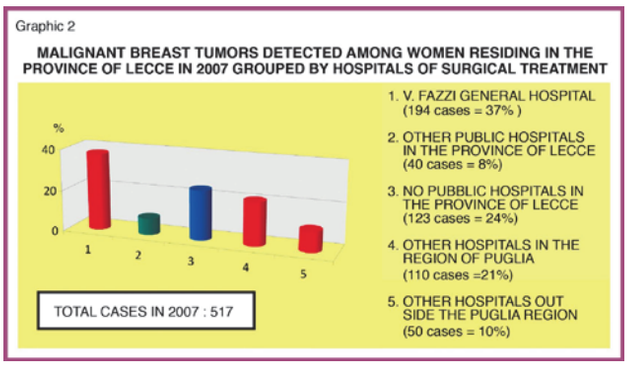
The aim of this study is to evaluate, by some GISMa's indicators, the quality trend of diagnosis and treatment of the mammary carcinoma in Salento over a period of ten years during which the PMS was introduced in Lecce's district (800.000 residents). The study is based on cases of breast pathology observed in resident women operated at "Vito Fazzi" General Hospital of Lecce, the biggest one in the district, that is home to breast unit and also reference center for diagnosis and for treatment of screen-detected cases of breast carcinoma in the PMS of the ASL of Lecce (local health authority). PMS in Lecce's district is active from 2008. The cases falling in the study were observed during the years 2003, 2004, 2010, 2011 and 2012 and were registered with SQTM. Their files have been analyzed in partnership with Piedmont's CPO. This work is part of a collaboration in monitoring program for mammographic screening in Italy, in which Lecce's team is so far the only one from Southern Italy. Preliminary data for the years 2003, 2004 and 2010 only were presented at the GISMa's National Meeting of Palermo in 2011 (Delos, Tarantino, Olla Atzeni et al. 2011) . The years 2003 and 2004 were evaluated for prescreening results. On the other side the years from 2010 were examined for any positive impact of the local PMS on the target population. With this work we hope provide useful informations to solve any critical issues of local PMS. From Cancer Registry of Lecce's district we can notice an incidence of about 500 breast malignant neoplasias a year in women (Melcarne, Rashid, Quarta 2010) .

Graphic 1



In the Graphic 1 we can observe breast malignant tumors, distributed by age groups, in the 2007, most recent year published available.

Graphic 2



In the Graphic 2, reporting the same cases distributed by hospitals of surgical treatment, we can observe that 37% of malignant breast tumors was operated at "Vito Fazzi" General Hospital of Lecce.

Materials and methods

This study concerns malignant and benign desease cases of the breast regarding women operated in the years 2003, 2004, 2010, 2011 and 2012 at Vito Fazzi General Hospital, where are treated surgically about 200 breast carcinoma cases for year, that is 37% of all breast malignant tumors cases incident in women of Lecce's district. All the cases were registered in SQTM by Surgical Pathology Unit of "Vito Fazzi" Hospital. For this first evaluation we haven't taken in to account all the indicators of the GISMa's surveys, but only those that usually can be deduced by the Surgical Pathology's diagnostic report. In particular for this study we have calculated with SQTM the values of the following indicators:

1) Pre-operative diagnosis in cancer (C5/B5)
2) Completeness of diagnostic and prognostic data in surgical pathology's report of invasive cancer cases (histotype, grading, pTNM)
3) Only one operation after pre-operative diagnosis of invasive cancer
4) Only one operation after pre-operative diagnosis of non-invasive cancer (in situ carcinoma)

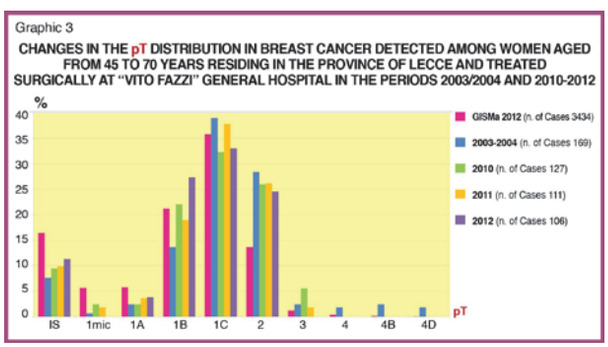
5) Axillary staging by Sentinel Lymph Node (SLN) only in pN0
6) No axillary dissection in Ductal Carcinoma in Situ (DCIS)
7) Conservative surgery (quadrantectomy) in invasive cancers </= 30 mm
8) Immediate plastic reconstruction in invasive carcinoma cases treated with total mastectomy
9) pN0 in invasive cancer cases
10) Early diagnosis (pTis + pTmc + pT1a + pT1b) in all cancer cases
11) Benign/Malignant ratio (B/M) in surgically treated cases

Indicators from 1 to 8 are GISMa's ones (Mano, Ponti, Angiolini et al 2013) . Indicators from 9 to 11 usually are not reported in GISMa's Surveys, but they are anyway calculated for this study and comparated with the corresponding values achieved from data of GISMa'Survey 2012 (Ponti, Mano, Tomatis et al. 2015).

Results

We can find in Table 1 the obtained values, reported as cases number and percentages distributed by years of treatment, for all the different indicators considered. The years 2003 and 2004 are reported together. In the same table are reported the corresponding values of the national GISMa's Survey 2012.

Graphic 3



In Graphic 3 we reported changes over time in the pT distribution in breast cancer detected among women aged between 45 and 70 years, who are the ones that benefit most from the PMS.

Indicator n. 1 increases from 17,2% of the prescreening period to the 29% for 2011 and to 23% for 2012. Indicator n. 2 increases from 31% to 74,7% in 2012. Indicator n. 3 remained nearly the same only with little variations over time. Indicator n. 4 gets better, from 61,5% of 2003-2004 to 87,5% of 2012. Indicator n. 5 increases from 29,5% to 63,6%. Indicator n. 6 doesn't improve so much, but it has little variations (from 76,9% to 80%). Indicator n. 7 has improved only slightly over time (from 63,8% to 66,7%). Indicator n. 8 increased by 10% over time, but started from 0 in the prescreening period. Both indicators n. 9 and n. 10 improved a little, from 54,9% to 58,2% the first, from 31,3% to 37,2% the second. But in Graphic 3 you can see that pT distribution highlighted changes indicative of an earlier diagnosis in the years of PMS. In particular they are increased cases of carcinoma in situ, microinvasive carcinomas and carcinoma within 1 cm and decreased cases of carcinoma above 2 cm. Indicator n. 11, expressed as a ratio, has gotten a lot better, gradually improving from 1,41 of the prescreening period to 0,56 of 2012.

Discussion

The results of this investigation highlight an almost progressive and significant improvement of all the indicators over time from the prescreening period, that is years 2003 and 2004, when in the Lecce's district the early diagnosis for mammary tumors wasn't planned or organized but it only depended on women themselves. In details we want to underline, first of all, that the improvement of indicators 9 and 10 is expression of a meaningful, although initial, effect of PMS in diagnostic earliness. Over time, in effect, in years of PMS we diagnosed more mammary neoplasias of little dimensions and that doesn't affect axillary lymph nodes than in the prescreening period. Anyway the recorded improvement of these two indicators is not satisfactory yet, being their values distant from GISMa's values of 2012 (72,6 and 49,7 respectively), although during 2011 they got nearer (70,2 and 38,8). But we have to pay attention to the fact that GISMa's values are representative of geographic areas of Northern Italy where PMS, in activity from 90's, are the re-

sult of decades of experience and organization that allows higher levels of coverage and compliance, as well as a quality of diagnostic and therapeutic courses certainly superiors than in Lecce's experience that was just starting in the years of this study. We think however that this first result of PMS in Salento, talking about more precocious diagnosis, as highlighted by moderate improvement of indicators 9 and 10, is important and indicative about what potential can be developed over time with an implementation of screening activities. Particularly positive was the result of indicator n. 2, typical process indicator, that recorded progressive growing values until it goes over the medium GISMa's survey value (74,7% versus 62,6%). This result attests the elevated level reached in the ASL of Lecce by the breast surgical pathology, that is essential base for appropriateness and quality of medical care. About the appropriateness for the surgery treatment, we have used the indicators from number 3 to 7. Their monitoring is useful to pursue a double objective, on the one hand, of avoiding useless and more invasive surgery in favor of the patients and, on the other, of saving costs and operating room resources. The results regarding the surgical appropriateness were contradictory. If for the indicators 3, 4 and 6 we had remarkable values, that improved significantly on time, aligning in 2011 to GISMa's values, indicators n. 5 and 7 were disappointing (63,6 and 66,7 respectively), remaining far below of the GISMa's values (91,7 and 83,5). Essentially it seems like the conservative surgery is still inadequately applied, in particular, regarding quadrantectomy instead of total mastectomy, as in effect is highlighted by indicator 7, which improved a little during years of PMS. Regarding the saving of axillary dissection in pN0 cases (indicator n. 5), the sentinel lymph node technique was still underused, although this parameter significantly improved if compared with the value (29,5%) of prescreening period. But the most critical aspects are those remarked by indicators n. 1, 8 and 11. Very negative is the result of indicator n. 1, for the systemic implications on all the diagnostic and therapeutic course and, in particular, for its adverse effect on appropriateness of surgical treatment. The value of this indicator improved very little, from 17,2% to 23%, remaining far below of the GISMa's value (82,3%). Despite this negative result we had positive values of indicators 3, 4, 6, as mentioned above, but this implies a systematic trend to bypass the pre-operative diagnosis by fine needle aspiration and/or needle biopsy, in favor of intra-operative diagnosis based on frozen sections. This practice is inadequate for several reasons and seems to be the heritage of the past when the surgeon was the only and almost exclusive reference for the breast pathology treatment. This practice should be abandoned in favor of the current good clinical practice recommendations because it causes the following negative consequences:

1) Increased costs due to overuse of intra-operative diagnosis, more expensive than the pre-operative one, as it lengthens the operating room time, requires the commitment of the medical and technical staff of surgical pathology during operations, brings to the operating table, as the indicator 11 remarks, many benign lesions, with a further consumption of resources and exposition to risk and discomfort the women unnecessarily operated;
2) lowering of the quality and appropriateness of the treatment plan due to bypassing the multidisciplinary assessment in pre-operative phase, leaving every decision to surgeon;
3) Exclusion of woman/patient from a decision regarding the treatment because she is under anesthesia when a decision is taken after a intra-operative diagnosis: the consequent implications, also talking about legal validity of consent to the treatment, can be easily understand.

About the very low value of indicator n. 8, regarding the plastic reconstruction of breast after total mastectomy, in part it's due to the multidisciplinary planning deficits of the diagnostic-therapeutic courses and to underuse of the pre-operative diagnosis.

Conclusions

From the results described above, albeit relative to a still initial period of introduction of the PMS in the district of the ASL of Lecce, with

the consequent and deducible limits both in terms of effective coverage of the target population and compliance of the invited women who appeared to be low (around the 40%), emerges already the capacity of this important early diagnosis planning tool. But also they emerge several critical issues that are attributable mainly to a deficit with regard to the approach and organization of multidisciplinary preoperative diagnostic and therapeutic planning process. These problems must be resolved through the efforts of medical teams involved , the Breast Unit, the body of the local PMS coordination and the management of the local health institutions. As the persistence over time of these critical issues , and still others in this study were not examined , produces the effect of devaluing the screening program, it is important to implement a systematic monitoring of the results with appropriate tools, to constantly correct the shooting of PMS and improve the quality of diagnostic and therapeutic path of breast cancer in the ASL of Lecce district.

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Biopolitics, Risk and Organization in Health Care

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Abstract

The study and analysis of the organization in health care depends on some aspects that include the observation of social phenomena, law categories, and political strategies as well as the administrative behaviors. All these aspects have led to the overcoming of the traditional concept of bureaucracy, which finds a solid theoretical foundation in the studies undertaken by Weber. In the Weber's vision, bureaucracy is the organization of people and resources for a collective purpose, public, according to any criteria of rationality, impartiality and impersonality. The assumption is that it is hard to perceive organizations oriented towards an end in a rational way, unless as bureaucracies, even considering that there may be non-bureaucratic organizational forms, not rationally oriented to a purpose (Weber 1922). One of the most original contributions of the late twentieth century comes from Luhmann's theory of social systems that is applied to the concept of organization. It provides an understanding of the object that goes beyond tautological assumptions (e.g. the organization is composed of men) and that permits to talk about organization as autopoietic system, not "closed" but "operationally closed" and therefore independent on the structural and operational plan (Luhmann 2000, 29-30). In the theory of systems - although the organizations may arise freely – what is defined as "complex" organizations, are formed within functions systems distinguished in economic organizations, political organizations, trade union organizations, health care organizations, etc. Main features include the possibility that they have to communicate with other systems in their environment (Luhmann and De Giorgi 1994, 328) and the reduction of uncertainty and risk. The absorption of uncertainty occurs when an organization elaborates some decisions that are a prerequisite for other decisions. In the social systems theory, all the organization activities are classified as decisions, take place at a given time, and are always documented. However, the evolutionary path that is used to trace the organizational profiles of complex apparatus cannot ignore the importance of Kuhn's theory, according to which scientific revolutions are characterized by the transition from one paradigm to another (Kuhn, 1999). The application of this latter model to the public organizations, permits to understand that even these organizations are subjected to the dynamics of cultural paradigms, according to which the transition from one paradigm - that no longer recognizes the organizations themselves - to another one, that rather includes new models, methods and practices, definitely involves a revolution (Limone 2008, 17). In order to properly talk about organization and e-government, especially in the health and medical sector, we first need to verify the most suitable organizational context able to manage innovation solutions and, therefore, analyze the organizational conditions as a prerequisite for the technological conditions. In fact, if the organizational environment does not respond to concrete parameters such as transparency, efficiency and economy, even the same e-government process is likely to fail. That is the only viable way to rationalizing and improving the public organizational system, such as health care systems, that tend to have high level of complexity and risk due to their nature.

Keywords: organization; risk; health care; integrated governance

1. Introduction: historical and evolutionary trails

The organization of the current complex structures - in particular the organization of health care facilities - depends on some aspects that include the observation of social phenomena, law categories, and political strategies as well as the administrative behaviors. All these aspects have led to the overcoming of the traditional concept of bureaucracy, which finds a solid

theoretical foundation in the studies undertaken by Weber.

In the Weber's vision, bureaucracy is the organization of people and resources for a collective purpose, public, according to any criteria of rationality, impartiality and impersonality. The assumption is that it is hard to perceive organizations oriented towards an end in a rational way, unless as bureaucracies, even considering that there may be non-bureaucratic organizational forms, not rationally oriented to a purpose (Weber 1922).

The Weber's studies have been developed later on through the contributions of Merton, although with different epistemological assumptions. Merton envisions his model by performing an ambivalent step: a) on one hand, he criticizes the rationality concept of bureaucracy in the Weberian model by using a functional approach; b) on the other side, he retrieves some elements of Weberian analysis for making criticism on his ideal model. In addition, Merton makes a distinction between the overt functions and the latent functions. Only with the analysis of latent functions, he shows that the Weber's model contains some sources of irrationality even with respect to the purpose, which do not consist in deficiencies in organizational design, but in the unforeseen effects that the pressure exerted by structures can provoke on the personality and behavior of men (Merton 1968).

In the seventies, Gouldner makes a change in the rules of Merton's scheme in tayloristic terms: he adopted a critical functionalism on the distinction between manifest and latent functions, able to identify the latent functions of measures, norms and institutions (Gouldner 1973). He identifies three key regulatory models of the bureaucratic action: a) the apparent bureaucracy, which occurs when both the directors and the employees have indifference attitude towards the respect of a rule imposed by an outside authority; b) representative bureaucracy, which occurs when both the directors and employees agree on the usefulness of observing certain rules; c) the taxation bureaucracy, which occurs when the rules are imposed from one side against the will of the others (Bonazzi, 2008, 232-235).

The Selznick's theoretical model, developed through a structural-functional analysis (*critical functionalism between Parsons and Merton*), shows a theoretical method that is intended to have general validity for any formal organization which has an internal bureaucracy. In particular, he introduces *The institute of cooptation* (defined as the process of absorption of new elements in the structure that determine the organization policy, as a way for preventing threats to its stability or existence) and performing, then, a further distinction between formal cooptation and informal or substantial cooptation (Selznick, 1969).

Crozier is primarily interested in some aspects such as safety, regularity and the impersonality of the functioning that are only found in the public administration entities. The issue that arises regards the functioning of such organizations and the social relations that exist within it, moving away from the post-Weberian functionalisms when they consider the difficulties of the change in bureaucratic organizations and when they highlight the relationship between technological innovation opportunities and the growing autonomy and cultural sophistication of individuals. In particular, (Crozier 1994, 22-27) Crozier carries out a pejorative interpretation of bureaucracy term: he conducts a strategic analysis of bureaucratic behaviors and defines power as control of uncertainty, highlighting the importance of National cultural models (Bonazzi 2008, 266-273).

In the eighties, the overcoming of the traditional bureaucracy was mainly defined by the managerial literature attributed to the studies of Drucker and Mintzberg, in which the "management by aims" model proposed by Drucker (Ivi, 287), may be viewed as specular antithesis of the traditional Weber's conception of bureaucracy. In this model, they provide an open debate beyond the hierarchies; the identification and negotiation of aims; the personalization of social relations; the acquisition of skills on the field; mobility in the career path and the majority attention given to the purpose rather than to the norms, as well as a form of "competitive democracy" at the workplace (Ivi, 292). All these factors result in a complete reversal of the axioms of the traditional Weberian model.

One of the most original contributions of the late twentieth century comes from Luhmann's theory of social systems that is applied to the concept of organization. It provides an understanding of the object that goes beyond tauto-

logical assumptions (e.g. the organization is composed of men) and that permits to talk about organization as autopoietic system, not "closed" but "operationally closed" and therefore independent on the structural and operational plan (Luhmann 2000, 29-30). In the theory of systems - although the organizations may arise freely – what is defined as "complex" organizations, are formed within functions systems distinguished in economic organizations, political organizations, trade union organizations, health care organizations, etc. Main features include the possibility that they have to communicate with other systems in their environment (Luhmann and De Giorgi 1994, 328) and the reduction of uncertainty and risk. The absorption of uncertainty occurs when an organization elaborates some decisions that are a prerequisite for other decisions. In the social systems theory, all the organization activities are classified as decisions, take place at a given time, and are always documented.

However, the evolutionary path that is used to trace the organizational profiles of complex apparatus cannot ignore the importance of Kuhn's theory, according to which scientific revolutions are characterized by the transition from one paradigm to another (Kuhn, 1999). The application of this latter model to the public organizations, permits to understand that even these organizations are subjected to the dynamics of cultural paradigms, according to which the transition from one paradigm - that no longer recognizes the organizations themselves - to another one, that rather includes new models, methods and practices, definitely involves a revolution (Limone 2008, 17). In order to properly talk about organization and e-government, especially in the health and medical sector, we first need to verify the most suitable organizational context able to manage innovation solutions and, therefore, analyze the organizational conditions as a prerequisite for the technological conditions. In fact, if the organizational environment does not respond to concrete parameters such as transparency, efficiency and economy, even the same e-government process is likely to fail.

The building blocks of the new paradigm reveal the need to pay attention to the reorganization of structures and internal functions (back-office) even before external activities (front office). The systematic intervention on the back-office ensures, in fact, that technological innovation is the same for the front office. That is the only viable way to rationalizing and improving the public organizational system, such as health care systems, that tend to have high level of complexity and risk due to their nature.

2. *Organization in health: risk and quality*

The concept of clinical governance was born in England, in the late nineties of the last century, within the politics of organizational strategies and regulatory system of the British National Health Service (NHS). The adoption of this concept comes from the first interventions on the quality management of health services that is meant as institutional duty shared among clinical professionals, experts in organization, health professionals and, in particular, policy makers. In the philosophy of clinical governance - according to NHS's guidelines largely accepted by our National Health System (SSN), organizations are responsible for the continuous improvement of the quality of their services and the safeguarding of elevated standards of care, through the creation of an environment in which the excellence in clinical care need to flourish (NHS, Department of Health, 1998).

The clinical governance can be considered as a new expression that may change the cultural system totally. In these terms, it provides resources to develop organizational skills oriented on sustainable health care, focused on patient, along with guaranteed quality that is necessary for the different stakeholders. In this perspective:

· Patients needs are in the spotlight of clinicians and administrators, who take shared responsibility,

· Information related to the quality of services are available to professionals, patients and the public,

· Differences in performance access, care processes and clinical results are measured with the continued commitment to reduce them,

· All organizations work together to continuously improve service quality,

· Professionals work as a team to deliver better performance in terms of clinical outcomes and safety,

· The risks and dangers to patients are brought to the lowest level,
· Health care is based on evidence and on good clinical practice.

According to this approach, the concept of clinical governance implies that the management of power takes place inside and outside the formal decision tree; these decisions arise from the interaction between the various stakeholders. Any person involved in the process becomes the bearer of specific needs and expectations, different scale of priorities and different capacity of perception of the results obtained (Wright, Hill 2005, 22-23). However, it is interesting to observe that once it is stabilized the process of clinical governance, the problem becomes its integration with all the other elements that constitute the different aspects of the management of health care organizations. Integrated governance is a further concept introduced for the first time in England in 2003, with the document entitled: "Governing the NHS: a Guide for NHS Boards (NHS Appointment Commission, 2003). The aim of this document is the integration of the different sectoral systems of governance (health, clinic, financial, management, research and information) and delete the existing overlays, in order to standardize (harmonize) different basal processes. The need for an integrated approach is based on the recognition that working for sectors - in a not shared way - is scattered and unproductive, so it is necessary to develop a unifying methodology that helps the organizations to realize their mission and reach the objectives (Wall, Halligan, Deighan, Cullen, 2002).

The concept of integrated governance goes beyond the corporate governance that is defined as the set of rules and organizational structures by which companies are managed and controlled. Health facilities (especially public ones) are considered as a constellation of several complex systems: there is the system of hospital care and the one related to primary care, the system of professional clinicians, and the one of professionals in organization, the system of the most important centers and the one in peripheries (Wright, Hill, 2005, 25).

The management philosophy of integrated governance also includes risk management, a methodology employed in health care settings but derived from the financial sector and that, in essence, involves the management of all those risks that threaten the value of an organization and that involve different aspects and different dimensions of organizational phenomenon: strategies, market processes, financial resources, human resources, technologies. However, the application of this methodology in the field of health care, cannot collapse in the transfer *sic et nunc* of principles and techniques designed in the industrial sector, financial or of the ICT (Novaco 2004, 24), although it is clear that even a health care company must deal with many risks that go beyond a particular risk and encompassing any general risks that any organization, regardless of the sector to which they belong, must know how to manage in terms of total quality.

Literature on organization, mainly from North American, was concerned to provide the definition of quality in health care systems and to draw up specific models (Donabedian 1966; Devlin 1990; Charlesworth 1993, 25). In 1984, Maxwell developed a model that includes six fundamental dimensions aimed to obtain efficient and effective level of quality in health care: 1) access to services; 2) the significance of collective needs (of the community); 3) the practical effectiveness for the "person" (individual patients); 4) the fairness and impartiality in the treatment; 5) social acceptability of the service supply; 6) the efficiency and economy of the service rendered (Maxwell, 1984, 1470-1472).

The study of Maxwell can be considered suitable for responding to the following questions:

a. Is the service physically and temporally accessible to the persons to whom it is addressed (in terms of physical accessibility and time)?

b. Do services, processes and procedures reflect the community and individual needs?

c. Does every single service allow obtaining the benefits or providing desired outcomes for individuals or groups of patients?

d. Is the service provided in an unbiased manner between the various categories or groups of patients?

e. Are the conditions for the provision of the service, the level of protection of privacy, the communication grade with patients, families and assistance team satisfactory?

f. Are the resources employed in the processes and in the phase of supplying services, used without waste?
g. Do detailed rules for the provision of the service (and those who provide it) meet the security measures that have to minimize the adverse effects of a treatment?
In any case it is observed that - regardless of the model used - the priorities linked to the respective principles depend on the needs and expectations of the parties involved that, in the health sphere, correspond to patients (beneficiaries of a service or a specific treatment), to professionals (medical staff and social health), managers (management and administrative staff) and finally to who really pays the service apart from services and performance received (taxpayers) (Wright, Hill 2005, 5-6).
A particular aspect related to health services quality, concerns the management of total quality (*Total Quality Management*), defined as a continuous improvement of quality (*Continuous Quality Improvement*) when it addresses to an organizational effort aimed at improving the overall performance. The key principles that are at the basis of the total quality management, are realized when: (a) the success of the organization resides in the accession of all its components to the needs of those who benefit from the service (patients); (b) quality is a consequential effect of the production processes in which the causal interactions are complex but understandable; c) the personnel involved in the process is intrinsically motivated to work with dedication and keep ethically corrected behaviors; d) the use of simple statistical methods associated with a correct collection and analysis of data, can constitute an effective procedure for the identification and understanding of problems related to operational processes and identification of risks.
In conclusion, total quality management implies the focus on operations and on expected results, analysis and the consequent identification of the needs of patients, analysis of the variations in processes or in results, the existence of multifunctional working groups for identifying and resolving quality issues, the use of data collected in a systematic manner at any point of the *problem solving* process for high-priority issues, causes, possible solutions and changes, learning and continuous improvement, process management tools to increase the effectiveness of working groups such as, for example, flowcharts, cause and effect diagrams, brainstorming, benchmarking (Wright, Hill 2005, 8).

3. The clinical risk and the safety of the patient

A correct definition of risk comes from the analysis of risk within the chain of genesis of the damage, with the purpose to clearly distinguish the different phases that often, in common parlance, are confused by the use of generic terms. The strategy is to start from some definitions laid down by the *Occupational Health and Safety Management system* (OHSAS 18001: 1999). In particular, it is distinguished between: a) hazard, situation or cause that enhances the damage; b) incident, occurrence that may give rise to damage; (c) accident, unexpected event and unfavorable cause of a damage.
These terms describe the stages of the chain by which it is generated a damage: the hazard represents an existing danger, which becomes a potential source of damage when it overlaps with an activity (e.g. the routine activities of a department or a health care facility). Sometimes, especially in health care, the link between activities and danger is so narrow that they may not be readily cleaved. This overlap determines the possibility that the danger is translated into an adverse event and this probability is the risk (risk) that may give rise to an incident, followed by a damage (accident). What binds event and damage are often unpredictable and fortuitous factors and very large number of events, which occur in the health and in other sectors without bringing any significant damage, demonstrates the lability of this bond. If the management of risk, in the health sector, is related to systematic processes of identification, assessment and treatment of actual and potential risks, the goal is focused on increment in safety of patients, to the improvement of the *outcome* and the indirectly reduction of costs, with a consequent reduction of preventable adverse events. For this purpose, the health care organizations - as it happens in industries and in other sectors - should analyze adverse events by using rigorous investigation techniques, in order to remove the system errors that are at the basis of such events.

In U.S., the publication of the report: *To err is human: building a safer health care system* (Washington, Institute of Medicine, 2000) allowed the starting of a series of researches on human errors in medicine. The report outlines a comprehensive strategy among government, market, patients and health services that try to reduce errors in medicine by inviting the Congress to realize a National-popular center for safety of the patients, who develop new tools and systems needed to solve this problem.

This turning point significantly contributes to analyze the relationship between ICT and risk management as part of the more general situation regarding the quality and safety of services (Esteves, Joseph, 2008). ICT are, in fact, powerful tools to support the organizational structure, decision-making processes (*Clinical Decision Support System, Health Technology Assessment*) and the monitoring of risk governance processes *(Clinical Data Repository, Electronic Medical Record*) (Friedman, Halpern, Fackler 2008, 69-76). However, this approach must take into account the transition from a purely reactive system (management of non-compliance, emergency management etc.) to a predominantly pro-active and preventive system.

A further aspect is the increasing attention on safety at all organizational levels. Adverse events are undoubtedly a problem of quality of care, and to that extent, they have a purely clinical relevance, but also have economic and social implications linked to the costs incurred by the health care facility that cause a general problem, but not less relevant, that is the loss of confidence of the population against the health service.

In this perspective, the safety of patients, assumes an importance that involves all phases and aspects of the organization. The lack of integration between the different organizational levels or the predominance of some over others, determine the loss of essential components of clinical risk management that lead to a partial vision and therefore not fully reliable.

If it is true that the primary purpose of a health care company is the protection of the health of patients and population, it is also evident that the strategies of *risk management* must be mainly oriented on prevention and risk management in accordance with the principle of Ippocrate *primum non nocere* (Reason 2004, 25).

In recent years, the safety of the patients was placed as a priority of the health services in many countries and this centrality could not be attributed to the occurrence of particular events, but rather to the dissemination of reports and epidemiological studies relating to iatrogenic damage. In the face of this importance, the management of risk becomes the strategic function of *a learning organization* model, i.e. an organization capable of sharing its knowledge, learn through participation in the various experiences and improve through the dissemination of new knowledge and culture technical-professional that characterizes it.

The cultural approach to the safety of the patients has a relief that cannot be overlooked, especially if you consider its bond with what is the vision of "Error" on which it is based. The determinant is found, in fact, in the passage from a vision of the error, as cause of system failure, to the vision of error generated by the complexity of the system itself.

The scientific and technological progress, the exponential process of specialization of medical sciences and the increasing organizational complexity, contribute to the increase in medical errors, although it has increased the awareness of the rights over time and thus the demand for greater transparency, clarity, accessibility, intelligibility and safety (Gainotti, Poppi 2004, 61). The debate on the issue is very intense and rich of paradoxes, "on one hand, no century has known such overwhelming progress in biomedical treatments and pharmacological properties as the twentieth century […], and everything suggests that the increasing pace of innovations diagnostic, therapeutic and rehabilitative services will continue. However, now as never, the uncertainties and suspicions are deep and widespread in fields such as science, basic health practices and in the chance of healing, as well as to ensure equal health care practice for not guaranteed patients "(Ardigo, 1997). According to this claim, the success of medicine is at the basis of its own weakness. Nowadays, failures that occur in diseases that were incurable in the past, are no longer perceived as tolerable but become errors. In addition, the hard and visible dispute between patient and clinician in the health care environment is becoming physiological. Today, patients expect to be guaranteed in terms of security and they demand to be in-

formed about all the risks associated with clinical practice, but, at the same time, clinicians feel heavily the risk of having transparent communication and free of complaints. That causes a gradual distancing and an unfair degradation of the fiduciary relationship between the parties involved, a fragile "wall of silence" (Gibson, Sing 2003) that is often subjected to attacks by the media and the specialized press. These situations lead medical staff to assume precautionary behaviors and the recourse of the so-called "defensive medicine".

In conclusion, in the relationship between clinical risk and patient safety, the recall of a sociopolitical role of active dialogue in drawing up strategies means, for the health system, the recognition and the assessment of risk, investing resources, introducing evaluation systems and organizational practices for proper management and prevention, making public the results (Cavicchi 2007).

The consuetude to provide data and information regarding clinical practice or other achieved performance data, lead to an internal comparison able to certainly increase the knowledge in a logic of community *empowerment*. This may gradually transform the cultural model in a model primarily oriented to the reconstruction of the fiduciary relationship between clinician and patient, despite the obvious difficulties concerning the measurement of the *outcome* in health.

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