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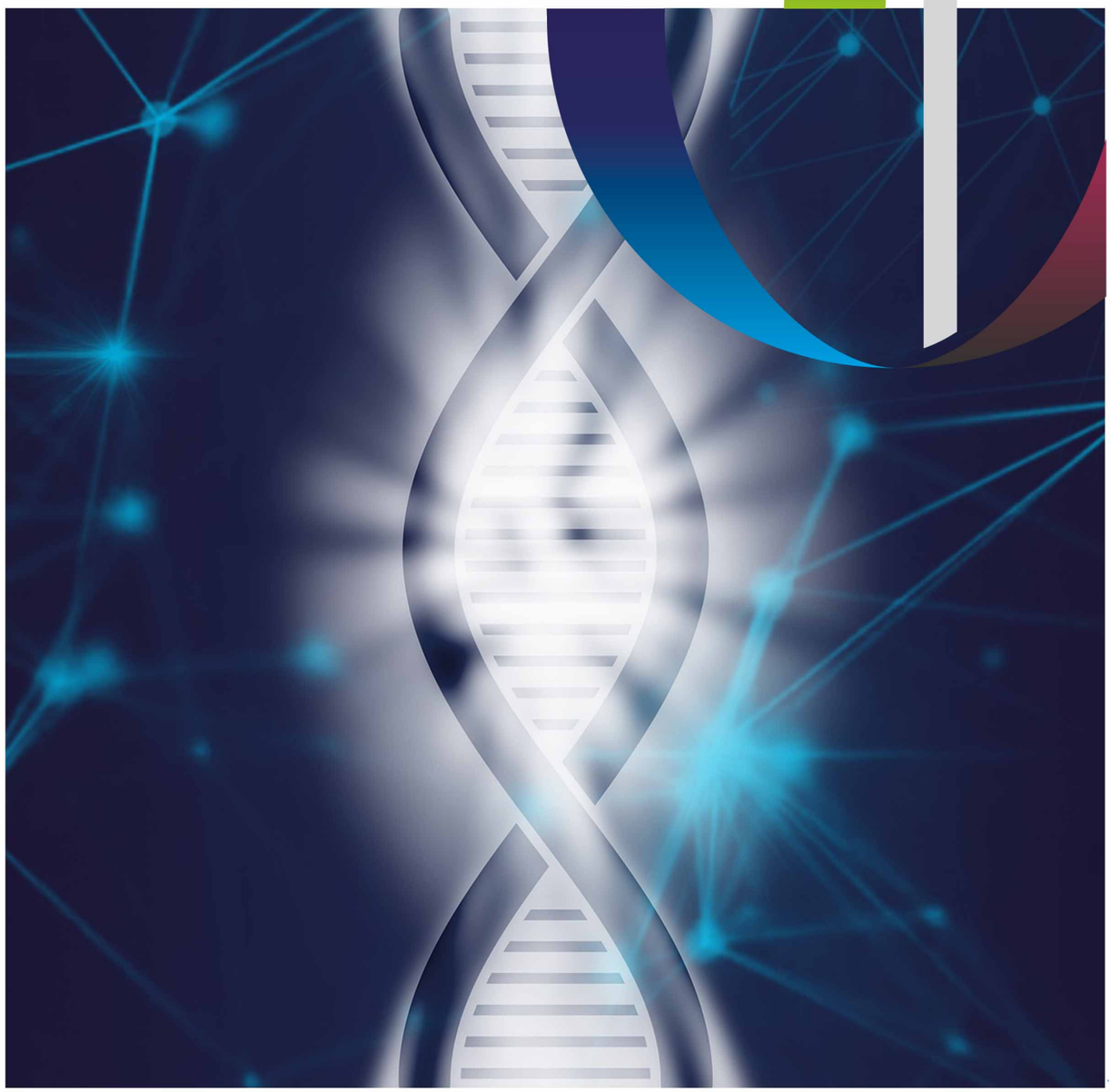

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UNIVERSITÀ DEL SALENTO

DREAM
laboratorio diffuso di ricerca interdisciplinare applicata alla medicina

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JDReAM

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

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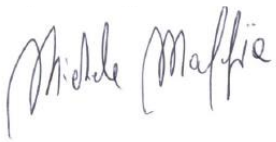

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Attitudes, knowledge and Practices of Nurses towards HIV/AIDS Patients. An Observational, Cross Sectional, Multicenter study

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Background. Attitudes, knowledge and practices of nurses towards HIV/AIDS patients are of continuous interest, especially in developing countries. However, in Italy, this topic is still scarcely debated.
Materials and methods. An observational, cross sectional, multicentre study was conducted on a sample of 144 nurses in two Italian Hospitals of the Puglia Region ("Vito Fazzi" Hospital in Lecce and "San Giuseppe da Copertino" Hospital in Copertino (Le)).
Results. A large part of the sample (97.2%, n=140) stated that they never refused to take care of a patient with AIDS. Only 22.9%, (n=33), of the sample had attended a training course and almost half (63.2%, n=91) used the gloves regularly when performing blood samples or when administering therapy to patients. With regard to the assessment of HIV knowledge, the percentage of nurses who know the meaning of the terms " seropositive" (83.3%, n=120), "HIV-positive person" (91.7%, n=132) and "window period" (47.9%, n=69) decreased.
Conclusions. The results of the study show that a non-negligible percentage of nurses could be considered at risk of infection due to non-routine use of gloves, incorrect handling of the patient's biological samples and not knowing how to decontaminate a surface with potentially infected blood.

Keywords: AIDS, HIV, knowledge, Attitudes, practise, HIV patients, nurses.

1. Introduction

The number of cases of HIV infection (Human Immunodeficiency Virus) has been steadily increasing in recent years. According to data provided by UNAIDS (2018) (https://www.epicentro.iss.it/aids/epidemiologia-mondo) there are 37.9 million HIV patients worldwide. In Italy alone 2,847 new infections have been reported, representing an incidence of 4.7 cases per 100,000 residents (Istituto Superiore di Sanità Istituto Superiore della Sanità 2019).
HIV is a retrovirus that attacks T lymphocytes, causing the death of these cells and a severe immunodeficiency of the individual who acquired the infection, called AIDS (Acquired Immuno Deficiency Syndrome), a chronic disabling disease (Waymack, Sundareshan, 2019) (Simon et al. 2006).

To prevent the disease from evolving, it is important to diagnose it early by detecting specific antibodies, antigens or both, using different commercial kits. On the other hand, the serological tests are generally used for screening. An important advance in this area has been the availability of rapid antibody tests for HIV-1, which are important tools for surveillance, screening and diagnosis. These can be reliably performed on plasma, serum, whole blood or saliva by healthcare professionals with limited laboratory expertise. Since 1981, when the virus was discovered, medicine has moved forward performing early identification of the virus in the body, so that treatment can be carried out immediately with combined antiretroviral therapy (CART) (Simon et al. 2006). This has greatly improved the quality of life of individuals affected by HIV, reducing the viral load and therefore the transmissibility of the virus, thus limiting the outbreak (SIMIT & Ministry of Health, 2017). However, current drugs do not eliminate HIV infection and permanent treatment may be necessary.

Despite the protection of the rights of people affected by HIV, the taboos that have been created since the discovery of the virus have influenced the thinking of society, causing difficulties in the lives of patients even in the simple daily actions and the quality of care that is provided to them. Studies conducted in the literature to analyse the behaviour and attitudes of nurses towards HIV + patients have shown that there is a very common tendency to be reluctant to provide services to them (Anderson et al. 2003) due to fear and anxiety of infection (Juan et al. 2004). By contrast, other studies have revealed a reduction in the discriminatory attitude of nurses especially when they are equipped with adequate material and essential protective equipment (Farotimi et al. 2015)

Studies conducted in Italy have shown that the majority of nurses (98%) would not refuse to take care of an HIV+ patient (Marranzano et al. 2013), although, at the same time, the respondents who contribute to producing the "stigma" are those working in the social and health sector. In this sense, in the Italian literature, there are few studies that have examined how the lack of knowledge is an obstacle to the effective provision of nursing services, so it is necessary to understand what factors favour stigmatizing attitudes in this context.

2. Aim of the study

Understand the level of knowledge, practices and attitudes of nurses towards people with HIV/AIDS.

3. Methods and Design

An observational, cross sectional and multicentre study was carried out between April and October 2019 at two Italian hospitals in the region of Puglia, in the province of Lecce. The study involved 144 nurses (n=144), with at least one year of work experience. The recruited nurses worked in different areas, in particular in the Medical Area (n=56, 38.9%), in the Critical Area (n=41, 28.5%), in the Surgical Area (n=27, 18.8%), in the Maternal-Infant Area (n=14, 9.7%) and in the Oncological Area (n=5, 3.5%). Those who were interested in participating were given an informed consent form, which reminded them about the voluntary nature of participation, as well as the confidentiality and anonymous nature of the information. A specially trained researcher explained the aims of the study to the facility managers. Each participant had 20 minutes to answer the questionnaire items. Subsequently, the completed questionnaires were placed in a sealed envelope to guarantee confidentiality and anonymity. In addition, to ensure that the questionnaires were anonymous and thus to allow identification of the participants, a sequential identification code (ID) was attributed to each participant. Each questionnaire, therefore, had an ID number which corresponded to the SPSS database ID.

3.1. Ethical considerations

The ethical characteristics of the study were set out in the questionnaire presentation. Participation in the study, being free and voluntary, was considered as an expression of consensus. It was specified that participation was voluntary and that the participant could refuse to participate in the protocol whenever he or she wished.

3.2. Tools

A questionnaire entitled "Knowledge, attitudes and practices towards patients with

HIV/AIDS" (Coniglio M.A. et al., 2013), previously used to assess the knowledge and attitudes of medical students towards HIV infection (Marranzano et al. 2013), was used for this study. The questionnaire consists of 26 items, divided into 3 sections.

The first section is used to collect social-demographic data and information on current and past work activity (items 1-6). The second section is composed of items on vocational training and years of work (items 7-12). The third section explores whether nurses have ever rejected an HIV patient, as well as practices and behaviours towards these patients, such as whether or not to use protective gloves during the handling of biological samples or during treatment. It also asked about the perception of the risk of contracting HIV (items 11-13.1). The last section tested the knowledge of HIV, in particular the meaning of "seropositive", "window period" (items 14- 24). The assessment of knowledge was made according to whether the answer was correct or incorrect. The non-response and "don't know" were classified as incorrect.

The questionnaire was administered on paper and anonymously to all nurses who gave their consent and had more than one year's work experience. Each professional had the freedom to fill in the questionnaire with respect for privacy, expressing their opinions and considerations on the subject.

3.3 Statistical analysis

Descriptive analyses were conducted for all qualitative and quantitative variables using the Software Statistical Package for Social Science (SPSS) version 17. The continuous variables were synthesized using mean and standard deviation (DS) and the categorical variables using frequencies and percentages.

4. Results

4.1. Socio-demographic characteristics of the sample

In the recruited group, the female gender (n=93; 64.6%) prevails over the male gender (n=51; 35.4%), with an average age of 42.28 years and a DS of 9.59.

As shown in Table 1, most of the sample has work experience between 21 and 25 years (n=31, 21.5%) and work in critical area (n=41, 28.5%) and medical area (n=56, 38.9%). The highest level of training achieved by most nurses is the Bachelor's Degree (45.8%), followed by those who have achieved the Regional Course (27.8%), the University Diploma (19.4%) and 4.2% and 2.8% have a Master's Degree and a 1st Level Master's Degree respectively (Tab.1).

Tab. 1 Social-demographic data	**N (%)**
1) Hospital	**144 (100%)**
"S. Giuseppe da Copertino" (Copertino)	18 (12.5%)
"Vito Fazzi" (Lecce)	126 (87.5%)
2) Age	
Media	42.28
Mode	47
Median	44.00
Standard deviation	9.59
Minimum	25
Maximum	62
3) Gender	**144 (100%)**
Female	93 (64.6%)
Male	51 (35.4%)
4) Civil status	**140 (97.2%)**
Cohabitant	21 (14.6%)
Divorced	6 (4.2%)
Single	32 (22.2%)
Married	78 (54.2%)
Widow	3 (2.1%)
5) Children	**144 (100%)**
No	59 (41.0%)
Yes	85 (59.0%)
6) Religious beliefs	**111 (77.1%)**
Agnostic	4 (2.8%)
Atheist	6 (4.2%)
Catholic	101 (70.1%)
7) Years of working experience	**144 (100%)**
1 - 5	21 (14.6%)
11 - 15	23 (16.0%)
16 - 20	21 (14.6%)
21 - 25	31 (21.5%)
26 - 30	10 (6.9%)
6 - 10	24 (16.7%)
Over 30	14 (9.7%)
8) Current working area	**143 (97.3%)**
Surgical area	27 (18.8%)
Critical area	41 (28.5%)
Maternal-children's area	14 (9.7%)
Medical area	56 (38.9%)
Oncological area	(3.5%)
9) Have you ever worked in other areas?	**144 (100%)**
No	25 (17.4%)
Yes	2.6%)
10) Higher level of education	**144 (100%)**

Regional course	40 (27.8%)
University Diploma	28 (19.4%)
Master's Degree	6 (4.2%)
Bachelor's degree	66 (45.8%)
Master 1st Level	4 (2.8%)

4.2. Practices, perception and knowledge towards HIV+ patients

With regard to the practices and behaviour of nurses towards HIV+ patients, an important fact is that 97.2% (n=140) stated that they have never refused to care for a patient with HIV. During clinical practice, 28.5% (n=41) of them only "a few times" use gloves to collect blood samples from HIV+ patients or to give them medication, and the reasons for this were: comfort or haste. On the other hand, 8.3% (n=12) do not use gloves "ever" (Tab 2).
Concerning the decontamination of a surface with potentially infected blood, half of the sample (53.5%, n=77) showed to be able to manage the situation correctly, using bleach (sodium hypochlorite) for 10 minutes, as recommended by the Guidelines for Biosafety in HIV diagnosis and research laboratories (WHO Global Programme 1991), against 19. 4% who could not answer the question and 27.1% (n=39) who answered incorrectly, indicating that 11.1% would use alcohol and 16% iodine.
On the other hand, with respect to the evaluation of knowledge related to HIV, the analysis showed that 77.1% (n=111) of the sample never attended a training course on this subject and 89.6% (n=129) would be interested in participating. Moreover, most of the sample (98.6%, n=142) are aware of the meaning of the acronym AIDS (Acquired Immunodeficiency Syndrome) and 96.5% (n=139) recognize the Immune System as the main target of the pathology. The percentage of nurses who are aware of the meaning of the term "seropositive" (83.3%, n=120), i.e. a person who has been infected with HIV and is therefore positive for laboratory tests, and of the meaning of "seropositive person" (91.7%, n=132), i.e. a person who is not ill and feels well for a long period of time after infection, is decreasing.
On the other hand, only 47.9% (n=69) know the meaning of the "window period", i.e. the time between potential exposure to HIV infection and the point at which the test will give an accurate result, compared to 8.3%, n=12, who did not answer the question, and 43.7%, n=63, who answered incorrectly. With regard to the opinion on who could contract AIDS, 29.2% (n=42) state that all people with risk behaviour can contract the virus, a category which also includes people with potential occupational risk, homosexuals and drug addicts. To the question "Is it possible for me to contract the infection from a person with AIDS?", the majority of the sample (86.1%, n=124) answered in the positive direction (Tab. 2).
Finally, when asked to describe the feelings and emotions felt towards a patient with HIV, the majority of the sample (20.8%, n=32) reported to adopt the same treatment between the HIV patient and the other patients, followed by 7.1%, (n=11), who replied to feel sorry towards these patients and 3.9%, (n=6), who reported to pay more attention when caring for them. Among the emotions most experienced, however, were anger (3.2%, n=5), fear and sadness (2.6%, n=4).

6. Discussion

The main objective of this study was to observe the level of knowledge, practices and attitudes of nurses towards people with HIV/AIDS.
With regard to the knowledge of the meaning of the acronym AIDS and being HIV-positive, our study showed better results than another study carried out in Israel, which in comparing the knowledge among Jewish and Arab nurses, only 72% of the former and 51% of the latter were aware of the meaning of the term "HIV-positive" (Azaiza et al. 2002, 331-339). The most solid knowledge with respect to HIV infection, therefore, concerns: the mode of transmission, the meaning of the acronym AIDS and being seropositive. The most lacking, instead, concern the "window period" (Azaiza et al. 2002) (Juanet al. 2004), (Marranzano et al. 2013).
Our data are in line with those present in a study carried out in Taiwan (Azaiza et al. 2002), in which nurses were tested on the basis of their knowledge and the score of correct answers was 71.6%, and another study carried out in Italy (Sicily), which showed a percentage of correct answers of 65% (Marranzano et al. 2013). With reference to training, it was found

that participation in AIDS Update Courses is very low (22.9%), but at the same time 89.6% of the sample would be interested in participating. This result differs from the national literature, which indicates 43% participation in AIDS seminars or updating courses (Marranzano et al. 2013).

With regard to the use of gloves in nursing practice, although the Ministerial Decree (1990) recommends the use of gloves in taking samples that are difficult to perform, due to the condition of the patient or the particularity of the sampling site (Ministerial Decree of 28 September 1990), in our study the percentage of nurses who use them regularly is lower than the standards found in (Marranzano et al. 2013), and different from the data obtained in a study carried out in Nigeria, where 95% would not use gloves in routine practices, except in the presence of HIV-positive patients (Farotimi et al. 2015). In addition, knowledge about methods of decontamination of a surface contaminated with infected blood was investigated. It was found that half of our sample (53.5%) would correctly use bleach (sodium hypochlorite 0.1%) to reduce the potential risk of infection, as recommended by the "Guidelines for Biosafety in HIV Diagnostic and Research Laboratories" (WHO Global Programme 1991), the other half, however, does not have adequate knowledge of how to decontaminate an infected surface. These results differ from those obtained in the study in Nigeria, where 65% of student nurses would decontaminate blood or body fluids with a 0.5% NaCl solution. (Farotimi et al. 2015). Theoretically, therefore, a non-negligible percentage of nurses in this study could be considered at risk for infection due to non-use of routine gloves, incorrect handling of the patient's biological samples, and lack of knowledge of how to decontaminate a surface with potentially infected blood.

7. Conclusions and Perspective

For a better care of the HIV patient we must have full control of the disease. Although the data from this study cannot be generalised to the general population, it may be the basis for future research, where we could also work on the experiences of HIV patients to better understand their care needs and avoid any stigma attitudes of health professionals. Factors such as misinformation, misguided care procedures or popular beliefs that could affect the quality of care provided to each patient have been analysed in the literature. In addition, as the National Plan of Action against HIV and AIDS 2016 (PNAIDS) makes clear, the full involvement of people with HIV in the development of the stigma reduction strategy should be encouraged. (National Plan of Action against HIV and AIDS 2016). In the fight against AIDS and all stigmatising attitudes related to it, the first "precaution" is the information and training of all health workers who interface with HIV+ patients on a daily basis, and who must ensure their safety, adequate care and respect for their human rights.

Tab. 2 Practice, perception and knowledge towards HIV+ patients	**N (%)**
11) Would you refuse to take care of a patient with HIV or AIDS?	**144 (100%)**
Absolutely, yes.	2 (1.4%)
No	141 (97.9%)
Probably yes	1 (0.7%)
12) Have you ever refused to take care of a patient with HIV or AIDS?	**144 (100%)**
No, never	140 (97.2%)
Yes, sometimes	3 (2.1%)
Yes, always	1 (0.7%)
13) Do you usually wear gloves to draw blood samples or to give medication to your patients?	**144 (100%)**
No, never	12 (8.3%)
Yes, sometimes	41 (28.5%)
Yes, always	91 (63.2%)
13.1) 13.1) If "yes, sometimes", why?	**27 (17.5%)**
It depends on the pathology	5 (3.2%)
Out of habit	1 (0.6%)
For convenience	9 (5.8%)
For distraction	2 (1.3%)
For haste	8 (5.2%)
For lack of the presidium	1 (0.6%)
For fear of infection	1 (0.6%)
14) Have you ever attended an updating course on HIV/AIDS?	**144 (100%)**
No	111 (77.1%)
Yes	33 (22.9%)
15) Would you like to attend a specific training course on HIV infection?	**141 (97.9%)**
No	12 (8.3%)
Yes	129 (89.6%)
16) Indicates the meaning of the acronym AIDS:	**144 (100%)**
Acquired immune deficiency syndrome	142 (98.6%)
Acquired Infectious Disease Syndrome	1 (0.7%)
I don't know	1 (0.7%)
17) AIDS is a viral disease responsi-	**144 (100%)**

ble for:	
Neuronal degeneration	1 (0.7%)
Destruction of the immune system	139 (96.5%)
Lung Diseases	3 (2.1%)
I don't know	1 (0.7%)
18) The term "HIV-positive" indicates that a person:	**144 (100%)**
He or she has been infected with the virus responsible for AIDS, so he or she is positive for laboratory testing.	120 (83.3%)
It has antibodies against the virus responsible for AIDS, so it is positive in laboratory tests and is protected against the disease.	17 (11.8%)
He has AIDS so he's positive for lab tests.	6 (4.2%)
I don't know	1 (0.7%)
19) An "HIV-positive" person:	**144 (100%)**
Since the beginning of the infection, he or she hasn't felt well.	4 (2.8%)
He looks like a sick person	4 (2.8%)
I don't know	4 (2.8%)
For a long period of time after the infection he is not sick and feels well	132 (91.7%)
20) The "window period" is:	**144 (100%)**
I don't know	12 (8.3%)
Time between potential exposure to HIV infection and the initial symptoms of the disease (AIDS)	50 (34.7%)
Time between potential exposure to HIV infection and the point where the test will give an accurate result	69 (47.9%)
Time between potential exposure to HIV infection and the point at which it can transmit the infection to another person	13 (9.0%)
21) When a surface is contaminated with potentially infected blood, it is necessary:	**144 (100%)**
Cover the surface with alcohol, dry and repeat the operation several times for at least 15 minutes.	16 (11.1%)
Cover the surface with bleach, dry and repeat the operation several times for at least 10 minutes.	77 (53.5%)
Cover the surface with iodine, dry and repeat the operation several times for at least 20 minutes.	23 (16.0%)
I don't know	28 (19.4%)
22) AIDS can be of interest:	**144 (100%)**
I don't know	2 (1.4%)
Homosexuals and drug addicts	14 (9.7%)
People with at-risk behaviour	42 (29.2%)
all the previous ones (homosexuals and drug addicts, people with risk behaviour, people with potential occupational exposure)	86 (59.7%)
23) Is it possible for me to contract the infection from a person with AIDS?	**144 (100%)**
No	12 (8.3%)
Yes	124 (86.1%)
I don't know	8 (5.6%)
24) Who wants to know if he is positive for HIV infection must:	**144 (100%)**
Carry out generic blood tests for infectious diseases	2 (1.4%)
Perform a specific test for HIV/AIDS	140 (97.2%)
I don't know	2 (1.4%)
25) Is there anyone among your relatives/friends who is HIV positive or has AIDS?	**144 (100%)**
No	115 (79.9%)
Yes	7 (4.9%)
I don't know	22 (15.3%)
25.1) Do you agree with:	**142 (98.6%)**
I don't know if I'm at risk of getting AIDS.	3 (2.1%)
I'm not at risk of getting AIDS.	4 (2.8%)
I'm at risk of getting AIDS like everyone else.	58 (40.3%)
I'm more at risk of getting AIDS than other people.	77 (53.5%)
26) Describe your sensations/emotions when thinking about an AIDS patient:	**92 (59.7%)**
Anguish	3 (1.9%)
Anxiety	2 (1.3%)
Compassion	3 (1.9%)
Understanding	4 (2.6%)
Sorry	11 (7.1%)
Contempt	2 (1.3%)
It is a person who has contracted AIDS by wrong behaviour or sometimes by his partner who, even though he knows he has it, has kept it hidden (and this is even more serious).	1 (0.6%)
Marginalisation	4 (2.6%)
More attention	6 (3.9%)
Fear	4 (2.6%)
Unlucky patient	1 (0.6%)
I think that it is not easy for the patient to lead a "normal" life because of the prejudices, behaviors and thoughts of some people in our society, so I still think of a person who is strong in his fragility.	1 (0.6%)
Little information	2 (1.3%)
Anger	5 (3.2%)
Feelings of attention and involvement, thinking about how he contracted the virus, which is often not caused by bad habits. The emotions the patient may have are very negative and often feel isolated. Patients should be considered as real patients and not as plagued.	1 (0.6%)
Solidarity	3 (1.9%)
Loneliness	1 (0.6%)
Hope for better treatment	2 (1.3%)
Same treatment between HIV+ patient and other patients	32 (20.8%)
Sadness	4 (2.6%)

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Monitoring Water Quality Using Plankton as Biosensor

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Abstract

In this paper we establish a baseline to use *Stentor coeruleus*, a freshwater single cell ciliate, as a chemical biosensor. We expose *Stentor* to an array of chemical species and concentration and monitored morphological and dynamic responses. We developed a computer vision pipeline to predict chemical exposure at sub-lethal doses. We present analysis for butylparaben, a common antimicrobial preservative used in cosmetics and food flavoring. Our preliminary results show high sensitivity of *Stentor* to sublethal chemical concentrations, amenable for use as an environmental biosensor when combined with the computer vision pipeline.

Keywords: Plankton, computer vision, biosensor engineering, image analysis

1. Introduction

Plankton is ubiquitous in our waters. From freshwater to seas and oceans, plankton represents the backbone of the aquatic food chain, as well as being a major actor in the bioregulation of local and global climate (Behrenfeld et al. 2005). The intimate connection between plankton and its local environment is apparent. Hence, the response of aquatic microorganisms to external perturbations can be used to make useful inference (Pastore et al. 2019).

Our objective is to combine a model organism with an image processing pipeline to produce robust and reliable assay for long-term monitoring chemicals in the aquatic environment. To determine the suitability of Stentor as a biosensor, we measure the morphological and dynamic responses to a variety of industrial chemicals and concentrations. We are particularly interested in sub-lethal doses, as lethal doses are trivial to determine (i.e. no movement).

In this paper we propose a system comprising a detector and a set of algorithms capable of establishing a link between external perturbations and morphological and behavioral modifications of plankton. The paper is organized as follows. In the first section we describe the experimental setup used for acquiring the videos of swimming plankton. Next, we discuss a feature engineering step, necessary to establish a relationship between the external perturbation and changes in the organism, as well as the algorithms applied for detecting plankton response to chemicals. Finally, we show the preliminary results obtained using the butylparaben and draw some conclusions.

2. Methodology

We used the single cells ciliate Stentor coeruleus as our test organism. The organisms were obtained from a commercial vendor (Carolina Biological Supply Company, Burlington, NC). For our preliminary work, we exposed the organisms to increasing concentration of bu-

tylparaben. We adopted a manual acquisition method that imaged our test organism in a single well of a 12 well tissue culture plate filled with a mixture of sterile spring water and butylparaben (Figure 1). We imaged Stentor using a 5 Megapixel camera module (Arducam) with an adjustable lens connected to a Raspberry Pi computer that captures 1080p resolution color video (1920x1080) at 30 frames per second. Eleven serial dilutions of butylparaben in sterilized spring water were prepared, from 971 ppm to 0.9 ppm. The wells were filled with live Stentor coeruleus hand-pipetted from the stock container. Each well contained an average of 10 Stentor in 0.5 mL of spring water. Each diluted butylparaben solution was added to one of 11 wells in the plate, resulting in a final concentration from 971 ppm to 1 ppb. In the twelfth well, 0.5 mL of sterile spring water was added for use as a control. The completed plate was swirled on a horizontal surface in a figure-eight pattern to mix the solution, then placed on the imaging platform (Figure 1). A one-minute video was captured for each well, starting with well #1 (the highest chemical concentration) and concluding with well #12 (no-chemical, control). All the Stentor in well #1 to #4 died within minutes of chemical introduction, establishing the highest non-lethal dose as 61 ppm.

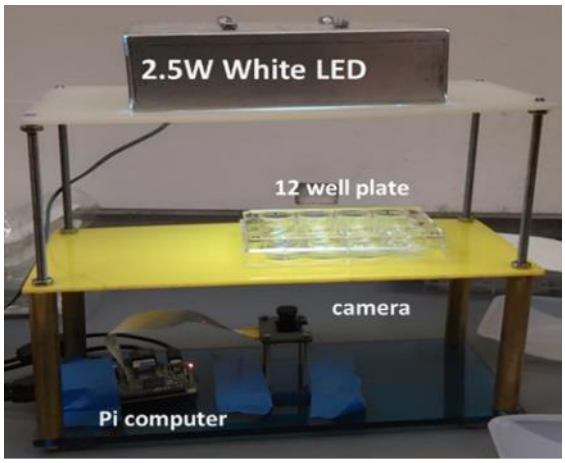
3. Image processing

The loaded wells contained a considerable amount of contamination (mostly algae), for it was too laborious to hand select individual Stentor for the chemical trials. Thus, videos required additional image processing to distinguish Stentor from the algae. Stentor were floating in a flat-bottom 17 mm deep clear polystyrene well and imaged from below. The tall vertical walls of the well optically obscured the perimeter of the well (Figure 1, middle row left) which often results in sample occlusion since Stentor tend to swim around the perimeter. The large diameter of the well reduced the resolution of each Stentor, contributing to the difficulty of distinguishing Stentor from algae.

The imaging processing pipeline consisted of detection, tracking, identification, feature extraction and analysis. The detection process converted the color image of each frame to a gray image, then converted the gray image to a binary quantized image. A tracking method placed a bounding box around each object in the binary image and measured the cell area by counting the number of "on" pixels in the bounding box. For each tracked object in each frame, the (x, y) center, height, width and area were stored. An identification method assigned a unique identification label (ID) to each object in a frame and maintained that ID across frames as the object moves. Images of algae were manually removed from the pipeline after the identification process.

4. Morphological features

Five classes of morphological features (Figure 2A) were extracted from the cropped plankton cell images captured in each frame of video. The classes consisted of 131 features (Figure 2B) (Vito P. Pastore et al. 2019). Each group of features capture different characteristics of the image. Shape-based features describe geometric aspects of the cell. Moments-based features have been shown to be useful in image classification and shape retrieval. Texture shape features include Haralick descriptors, Local Binary Patterns (LBP) and gray scale histograms. Fourier Descriptors (FD), extracted from the contours of the image, have been shown to be useful in differentiating shapes.



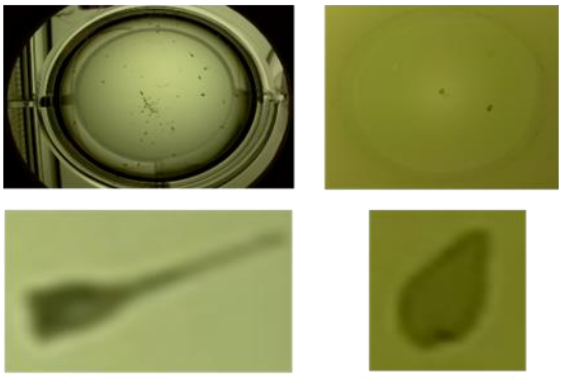
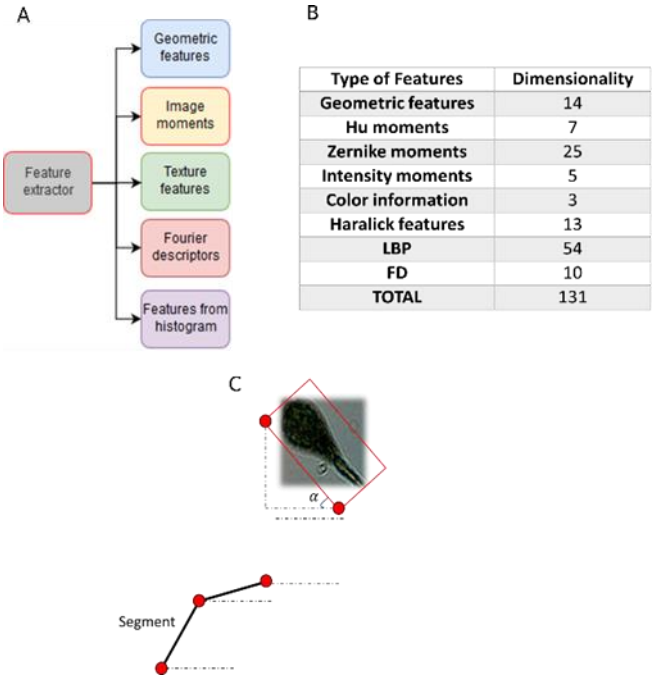


Figure 1: Experimental setup used for the acquisition of videos of swimming plankton exposed to chemicals. (top row) A 12 row plate of plankton is illuminated from above and imaged with a camera below. (middle row) A single well is imaged by the camera. (bottom row) Image processing tracks individual plankton cells.

5. Motion based features

We extracted four features from the Stentor reconstructed tracks. The average speed and body angle (Figure 2C) were extracted each frame. The body angle is defined as the angle that the major axis of the minimum fitting bounding box makes relative to the x axis (Figure 2C). A significant change in body angle indicates a change in direction. A segment length is the distance traveled between two body angle changes. The turning rate is number of turns occurring over the duration of a track.



Type of Features	Dimensionality
Geometric features	14
Hu moments	7
Zernike moments	25
Intensity moments	5
Color information	3
Haralick features	13
LBP	54
FD	10
TOTAL	131

Figure 2: Morphological and motion-based features extracted from the recorded videos. (A) Morphological features extracted by the feature detection module. (B) Number of dimensions for each feature sets used to calculate the morphological features. (C) Schematic overview for the behavior features extraction procedure

6. Multi-Target Tracking

Measuring the dynamics of multiple Stentor presents a multi-object tracking challenge (Bolme et al. 2010). The lack of unique identifiable features makes tracking difficult when Stentor cross paths. The presence of background noise (e.g. algae) creates cluttered space to search. Tracking involves detection followed by data association; linking the detections between two successive frames. Supervised learning is a useful technique detecting organisms. However, supervised learning requires a substantial training set and the large set of videos and resulting frames in our experiments makes manual annotation difficult.

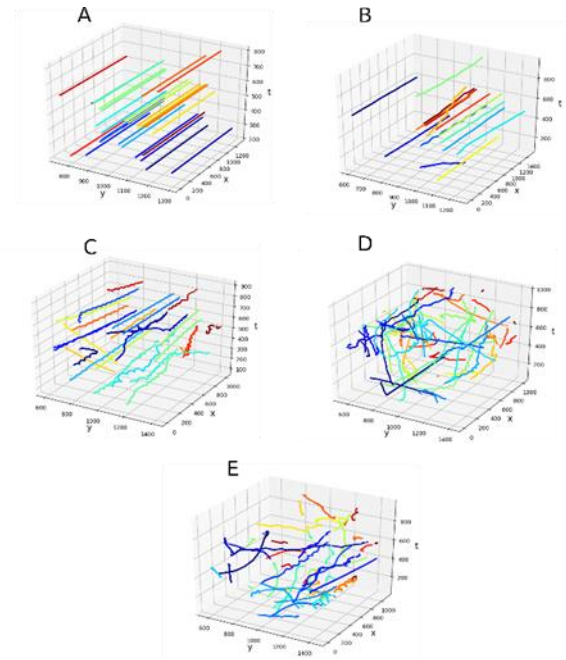


Figure 3: Tracking results of Stentor captured in 1000 frames (33.3 seconds) in decreasing concentration of Butylparaben. The axes are time (frame count), x and y position. Each colored trail represents the position of one Stentor. (A) At the highest concentration of 971 ppm, all Stentor are dead, indicated by the flat line trajectory (no motion). (B) At 121ppm a few Stentor process in place. (C) At 60.7 ppm a few Stentor are moving (D) At 3.8 ppm most Stentor are moving. (E) Without any chemical (control) all Stentor are moving.

To address this challenge, we implemented a semi-supervised annotation strategy. On the first frame, the tracker placed bounding boxes around each detected Stentor. For each successive frame, there were two possible outcomes: the tracker correctly tracked the Stentor, or the tracker incorrectly acquired another object (another Stentor or algae). When the latter outcome occurred, we manually correct the position.

The tracking training set was used to train an machine learning method that simultaneously tracks the Stentor and adapts to its changing appearance (Sujoy Kumar Biswas et al. 2019). The method uses random perturbation of Stentor images from the training set to learn a filter to recognize Stentor.

The tracking method produced a bounding box for each Stentor for each frame, along with a unique ID. The tracking output was used to identify motion patterns response of Stentor to each test chemical.

Figure 3E displays the tracks of the Stentor when they swim in the natural medium (control, no chemical). One can observe the random movement of the Stentor, indicative of food searching. However, the frequent changes in directions is noticeably subdued with the increasing dosage of the chemicals as evident in Figure 2 panels B-E. Ultimately the Stentor stop moving and die at toxic chemical concentrations. This is reflected by linear and straight tracks indicating the Stentor are barely moving or dead.

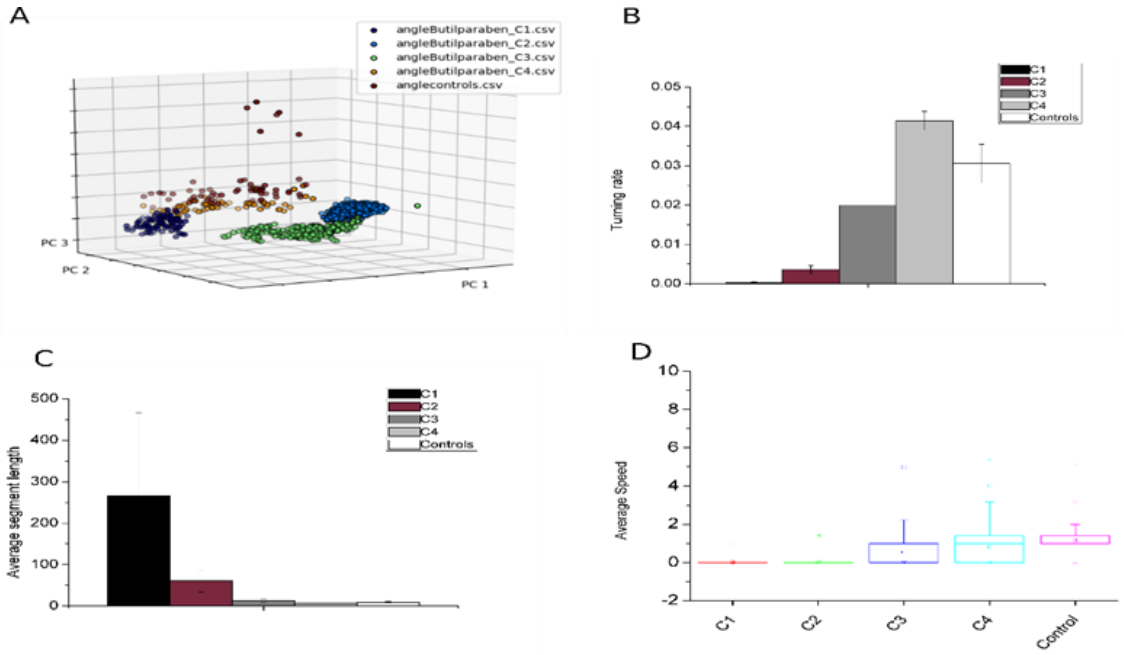
7. Morphological-dynamical analysis

We exposed Stentor to increasing doses of butylparaben. Figures 3A and 3B show that Stentor have mostly been incapacitated by the first two concentrations of chemical. As the chemical concentration is reduced, the swimming behavior resembles the controls (Figure 3E). We used the features described in Section 3 to visualize in the feature space of the Stentor under five chemical concentrations. We performed a Principal Component Analysis (PCA) and presented the results in Figure 4A. The PCA is computed using behavioral and morphological features, listed in Figure 2. The Stentor exposed to the highest concentrations (971 ppm, 121 ppm and 60.7 ppm) can be distinguished from the other conditions. When the concentration is sufficiently low (3.8 ppm) the correspondent cluster become close to the control cluster, while still being well separable. These results suggest that Stentor morphological and dynamical difference can be distinguished at sub-lethal chemical concentrations.

Dynamic analysis was conducted on motion features for each chemical concentration and control. Figure 4B shows the average turning rate of all the Stentor in well at four chemical concentrations and control. Figure 4C shows the corresponding average segment length. Figure 4D shows the average speed. Exposure to sub-lethal concentrations of butylparaben impaired Stentor movement, reducing their speed and turning frequency. This will have significant consequences to their survival as it adversely impact their ability to hunt food and avoid predators.

8. Conclusions

Our preliminary results show high sensitivity of Stentor coeruleus to low non-lethal doses of butylparaben. We demonstrated that the introduction of the chemical creates cell morphological and dynamic modifications in the feature space. High chemical concentrations kill or render them nearly motionless while sublethal doses adversely impact speed, turning rate and trajectory. The results suggest that the Stentor coeruleus is a good model organism for a chemical biosensor. Combining Stentor with a computer vision pipeline that implement feature detection and analysis may provide an automated method to monitor sublethal chemical toxicity in the aquatic environment. Further analysis is necessary to determine if Stentor response can be used to discriminate among common chemical pollutants found in fresh water.



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Osteochondroplasty tracheobroncopathy

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Abstract
Osteoplastic tracheopathy is a rare disease with dark etiology, characterized by the presence of nodular cartilaginous or bone nodular growths (masses and spicules) in the lumen of the tracheobronchial tree. According to some authors, these nodular outgrowths represent exostosis or eccondrosis of the cartilaginous trachea which often ossifies. Other authors relate this pathology to tracheobronchial amyloidosis. Clinical symptoms can be characterized by cough, dyspnoea, recurrent trachebronchial infections, occasional hemoptysis. Bronchoscopic examination allows the diagnosis to be made. The clinical cases in which the above described pathology has been diagnosed are three: 51 year old man smoker with chronic bronchitis, 62 year old woman smoker with moderate chronic obstructive pulmonary disease and a 65 year oldman, non-smoker, with severe obstruction entity.

Keywords: Osteochondroplasty tracheopathy, nodular cartilaginous or bone growths, tracheobronchial lumen, posterior wall of the trachea (pars membranacea) free.

1. Introduction

Osteochondroplastic tracheopathy is a rare disease of the trachea and bronchi that appears in the fourth-fifth decade of life, characterized by nodules or cartilaginous or bone spicules that project from the submucosa into the tracheobronchial lumen. It was first described by Wilks in 1837 and has been extensively studied from a clinical and pathogenic point of view by Balgaard in 1947. The etiology is highly uncertain. It seems possible that the connective cells of the tracheal wall can pose themselves to cartilaginous elements forming islands that will undergo partial calcification. These islands can also merge with tracheal cartilages. Some authors relate this disease to tracheobronchial amyloidosis due to the tendency of the latter to cause calcification or ossification. In this perspective, it could represent the terminal state of amyloidosis. According to other authors, this pathology is not the end result of amyloidosis and cannot be associated in any way with the latter. The endoscopic picture is typical for the presence of numerous cartilaginous and bony nodular growths (masserelle and spicules) that produce sessile or pedunculate endoluminary protrusions that give the trachea and bronchi a rosary crown appearance. The mucosa is intact and ulcerations that can cause hemoptysis are rarely found, in any case there is a marked rigidity of the wall. Tracheal obstruction can be mild, moderate and sometimes even severe. Over the years the cartilaginous and nodular protrusions tend to extend, thus affecting more and more vast trachea tracts and large bronchi; in more advanced cases they flow together forming plaques or masses which determine a marked rigidity of the wall and a considerable narrowing of the tracheobronchial lumen. Performing a biopsy is very difficult due to the hard consistency of the tissue, however bronchoscopy is diagnostic.

2. Clinical Cases

The first clinical case concerns a 51-year-old man, chronic bronchitis, smoker since the age of 15. He reported recurrent episodes of productive cough with sputum sometimes streaked with blood. Laboratory and respiratory function tests were normal. The same subsequently per-

formed bronchoscopy and chest CT scan. Bronchoscopy highlighted a picture of early stage osteochondroplastic tracheopathy, characterized by cartilaginous and bone nodules affecting the wall of the trachea, sparing the pars membranacea. The CT scan, on the other hand, showed a slight reduction in the size of the trachea which showed a modest deformation.

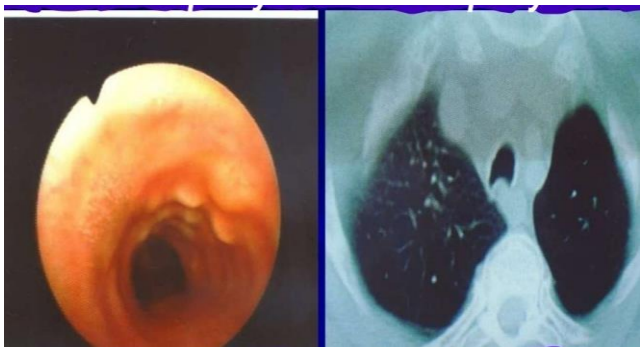

Figure 1. Presence of cartilage and bone tissue proliferation islands affecting the tracheal wall excluding the posterior wall.

The second clinical case that presented itself to me was that of a 62-year-old woman who smoked, suffering from chronic bronchopathy for several years. For about a week, she has been reporting an accentuation of the cough with the emission of mucopurulent sputum, exertional dyspnea and a minor episode of hemoptysis. Laboratory tests revealed only a slight increase in white blood cells, while lung function tests showed a modest reduction in respiratory function parameters (FV1 and FVC). The fibrobronchoscopic examination was characterized by the presence on the walls of the trachea (excluding the posterior wall, pars membranacea) of numerous nodular bony and cartilaginous growths which adorned the trachea as beads with a rosary crown appearance which in part flowed together forming plaques. In the light of this endoscopic finding, the diagnosis of osteochondroplastic tracheobroncopathy was made.

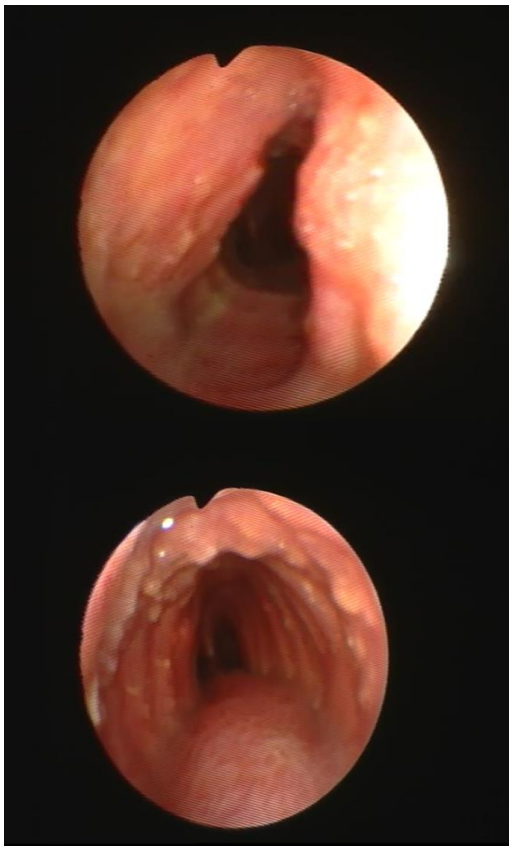

Figure 2 Nodular growths of the tracheal wall, with a rosary crown appearance.

Figure 3 Nodular growths partly confluent at the level of the tracheal wall that do not affect the pars membranacea(rear wall)

The third case report concerns a 65-year-old non-smoker man treated for chronic obstructive bronchopathy who reported that he had poorly productive cough and dyspnoea for many years. This blood gas analysis showed a reduction in P02 (60 mmHg). Bronchoscopy showed a significant reduction in size of the trachea and main bronchi due to the presence of plaques due to the confluence of nodular growths. Thoracic CT scan confirmed this reduction in size.

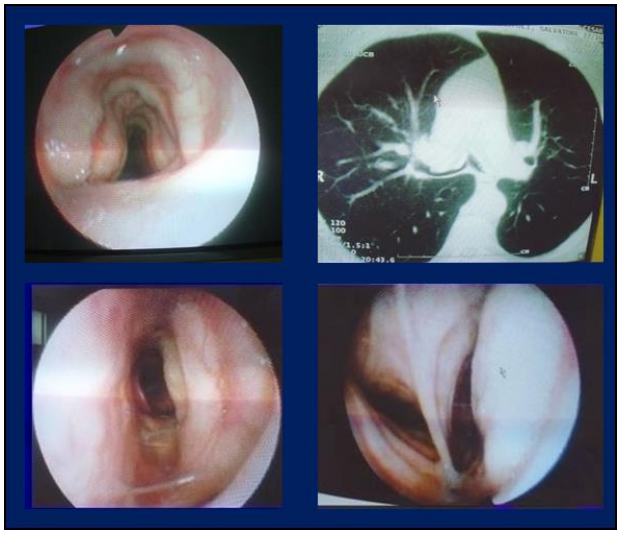

Figure 4 Stenosis of the trachea and large bronchi due to the confluence of nodular excrements that form plaques or masses

3. Conclusions and considerations

This is a very rare pathology characterized, as we have seen, by nodular cartilaginous and bony growths affecting the wall of the trachea (excluding pars membranacea) and bronchi. This pathology in the most severe form causes a significant obstruction of the trachea and large bronchi causing a symptomatology characterized by dyspnea with minimal effort. In these cases, however, the Yag laser treatment of the plaques or tracheal masses reduces stenosis and consequently improves the symptomatology.

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CESAREAN MYOMECTOMY TECHNIQUE: A CRITICAL REVIEW

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Background: Cesarean myomectomy (CM) is, for many gynecologists, a discussed surgical procedure characterized by fibroid's removal during cesarean section (CS) followed by possible complications. For a long time, it has been common and shared thinking that myoma should not be surgically treated in pregnant women at the time of birth through CS in order to avoid complications for the patient. Nowadays, many studies reconsidered the feasibility of CM. The aim of this investigation is to summarize the scientific evidences on this procedure, highlighting when and how it can be performed safely.
Methods: This review has realized basing on evidences reported in scientific database, as PubMed, Scopus, Cochrane Library, Medline and EMBASE databases, found using key words of reference to the main topic, the cesarean myomectomy. All the observational studies and meta-analysis published in the current century has been considered and results were critically evaluated by the authors, experts on CM.
Results: According to the findings reported, the CM could be a valid and feasible treatment option for patients, especially when there are specific conditions which are associated with a lower risk of complications, as perioperative blood loss and risk of hysterectomy. Authors' experience and judgment agree with the evidences about the importance of the surgeons' skill and experience, both to make safer the surgical procedure and to have intraoperative and post-surgical outcomes.
Conclusion: The applied surgical technique and the informative counselling and/or informed consent, still need standardization for a safer CM, both for the patients and for the operator, at the same time.

Keywords:
Cesarean Myomectomy, Cesarean Section, Myoma, Fibroids, Pregnancy, Complications.

1. Introduction

In the last decades epidemiological data have reported a growing rise of the average pregnant age: This phenomenon hits mostly industrialized countries, where the percentage of women giving birth to a child in their 40's has more than doubled to date (Bellieni 2016). Postponing pregnancy and delayed childbearing have brought to light problems of management of coexisting organic diseases. The incidence of such pathologies is directly linked to the age, therefore obstetricians have had to face new clinical questions on topics less frequent and less investigated in the past. This lead to reevaluate and subvert ancient axioms and preconceptions, bringing out an innovative obstruct point of view: the caesarean myomectomy. This surgical procedure is currently discussed for the risk of complications during and after surgery. Myomas or leiomyoma, also known as uterine fibroids, are the most common benign lesions

in women, prevalently consisting of monoclonal smooth muscle cell. Fibroids' presence during pregnancy is often associated with a high risk of cesarean section, as well as peripartum hemorrhage (Laughlin, Schroeder, and Baird 2010; Sparić 2014; Klatsky et al. 2008). The prevalence of myomas ranges between 20% and 25%, while their incidence can be up to 70% in women of reproductive age ('Myomas and Reproductive Function' 2004; 'Myomas and Reproductive Function' 2006).

2. Epidemiology of fibroids' in pregnancy

According to increase of maternal age mentioned above, the prevalence of fibroids during pregnancy has been increasing too during these years, shifting from 0.1%-3.9%, as reported in the initial reports, up to 10.7% in the most recent evidences (Cooper and Okolo 2005; Exacoustòs and Rosati 1993; Hartmann et al. 2009). Considering that these data are based on ultrasound assessment during pregnancy, it could be considered underestimated, because only 4% of myomas in pregnant women are diagnosed by ultrasound (Svigos, Robinson, and Vigneswaran 2006) and so many of them are detected mostly during cesarean delivery (Exacoustòs and Rosati 1993; Hartmann et al. 2009). This is also the reason why the choice of removing of myomas during cesarean delivery is not always an elective and premeditated one, but often it is made impromptu at operating table. Therefore, it is important to have good scientific evidences that can correctly guide the management of these lesions during pregnancy and mostly at the time of childbirth in order to guarantee the best outcomes for the patient. Nevertheless, cesarean myomectomy seems to be not yet sufficiently studied, this surgical procedure has its roots in the past.

3. History of cesarean myomectomy

It was reported in literature for the first time at the beginning of the twentieth century, when Bonney successfully removed up to six fibroids without any complications for the patient, who had three subsequent uncomplicated vaginal deliveries (Jauniaux and Khan 2014). Several other attempts followed in subsequent years, many of which were uneventful, but at the same time emerges a series of complications which apparently have a higher incidence in this type of procedure rather than cesarean section only (Lolis, Zikopoulos, and Paraskevaidis 1994). Finally, cesarean myomectomy was strongly discouraged also by worthy textbooks (Park and Kim 2009; Sparic et al. 2014), as Te Linde's too ('Te Linde's Operative Gynecology; Mattingly RF 1977).

Despite of this, the choice to avoid fibroid removal in pregnant women at the time of childbirth is not altogether a safer choice (Davis et al. 1990; Kwawukume 2002). In fact several studies are trying to attest the feasibility of cesarean myomectomy, because, as well as supported by many obstetricians, performing this apparently hazardous procedure through a safer way allows, on the other hand, in a single surgical time, to reduce other risks related to myoma in pregnancy and delivery (Klatsky et al. 2008; Park and Kim 2009), like:

-fertility impairment, miscarriage, placental insertion anomalies, alteration of fetal growth, fetal malpresentation, preterm birth and dystocia for subsequent pregnancy;

-peri-partum hemorrhage at the time of delivery with persistence of fibroid in situ;

-uterine rupture during pregnancy, in case of previous myomectomy.

Especially the latter represents two severe conditions burdened by a high rate of morbidity and mortality. In cases of previous myomectomy, the onset of a uterine rupture at the site of the scar during pregnancy or in labor is an occurrence which happens 0.2%-3.7% in an unpredictable way. According to some authors, uterine rupture is not always related to the length of interval between the fibroid removal and conception, as a matter of fact there is not an ideal interval which completely reset this risk (Gambacorti-Passerini et al. 2016). Moreover, the presence of one or more myomas, especially when they are large and/or intramural located, can hinder myometrium contractility mostly immediately after delivery and afterbirth, facilitating the onset of a life-threatening condition, like postpartum hemorrhage (Davis et al. 1990; Park and Kim 2009).

4. Fibroids' management during delivery

Fibroid removal during cesarean delivery could be an option to promote the physiological hemostatic effect of uterine contractions, making postpartum safer as far as possible, on the basis of modifiable etiological factors. Paradoxically, the most feared complication mentioned for cesarean myomectomy is just massive blood loss, which can happen intra and post-partum and which can be sometimes the reason for a hysterectomy. What lead many obstetricians to opt for fibroids removal during cesarean delivery, rather than leaving myomas in place, is that the recent experience on this surgical procedure has even more shown the possibility to reduce the hemorrhage risk, after taking specific precautions and techniques (Qidwai, Caughey, and Jacoby 2006; Cunningham et al. 2001). Furthermore, a really relevant benefit of cesarean myomectomy rather than myomectomy following pregnancy or the other way around is that a single surgical time significantly reduce costs and anesthetic complications, resulting more feasible in terms of cost and benefits (Awoleke 2013; Lolis, Zikopoulos, and Paraskevaidis 1994)).

According to recent studies performing a myomectomy during a cesarean section instead of on non-pregnant woman should be more suitable and advantageous for the surgeon as well as for the patient herself for several reason (Sparić et al. 2019; Hatırnaz et al. 2020; Tinelli et al. 2014). First of all, considering both that, contrary to what was thought in the past, myoma's dimension does not significantly varies during pregnancy (Muram, Gillieson, and Walters 1980). The volume of the uterine mass growths proportionally to amenorrhea (Shynlova et al. 2009) and fibroid is however relatively smaller than pregnant uterus. Therefore, the uterine incision needed to remove fibroid will be respectively always smaller during cesarean myomectomy rather than on a non-pregnant uterus, favoring wound healing with a smaller scar and so a lower risk of subsequent dehiscence or uterine rupture (Sparić et al. 2017). Moreover, during pregnancy, the gradual hypertrophy and hyperplasia of uterine smooth muscle cells, which develops also around myoma, facilitate the distinction between the lesion and the muscular layer, being more visible the cleavage plane. The easier removal of myoma reflects also the possibility to apply easier specific technique of myomectomy associated with lower risk of blood loss, like intracapsular myoma removing (Tinelli et al. 2014).

So that all this modification inevitably, contrary of what sustained in the past, can be considered the basis to reduce the risk of complications, making easier for the surgeon to use specific technique in order to limit the blood loss and other complications related to cesarean myomectomy.

5. Safety of cesarean myomectomy

The studies reported in literature on cesarean myomectomy are not yet enough satisfactory by a statistical point of view in term of quantity and quality of the evidences in order to definitively establish the safety and feasibility of the procedure. As a matter of fact, scientific databases reported less than thirty observational studies on the subject in question and, to date, no randomized controlled trial. Moreover most of them are retrospective studies and in the current century only six are the prospective studies mentioned in literature, which were respectively conducted by Kwawukume, first, in 2002 (40), Li et al in 2009 (41), Lin et al in 2010 (42), Tinelli et al in 2013 (43) and the two published in 2017 respectively by Valson et al (44) and by Rai and Mishra (45). No further and more recent prospective studies on cesarean myomectomy are reported in literature. With the sole exception of the Li's study, for which a total of 1387 patients were enrolled, 1242 cases and 145 controls, the other prospective studies are characterized by a small sample, well below hundreds (Kwawukume 2002; Li et al. 2009; Lin et al. 2010; Tinelli et al. 2014), mostly the two more recent ones (Rai and Mishra 2017; Valson, Nazer, and Mukerjee 2017). Retrospective studies on cesarean myomectomy, although more numerous, are almost burdened by the same limitation (Pergialiotis et al. 2017) and, for this reason, the latest study conducted by Zhao et al in 2019 stands out among them, having so far the biggest sample of patients, 2565 women all in all, whom 2344 underwent cesarean myomectomy and 221 cesarean delivery alone

(Zhao et al. 2019). The dimension of sample and the nature of the studies are the most strong limitations which allows to let many obstetricians still doubt quality of the study results, although the common findings of all the them seems to direct towards rather than against cesarean myomectomy (Zhao et al. 2019; Pergialiotis et al. 2017; Guler et al. 2020; Nargis, Karim, and Loverine 2019; Sparić et al. 2019, 2018; Senturk et al. 2017; Hatırnaz et al. 2020; Kwawukume 2002; Li et al. 2009; Lin et al. 2010; Tinelli et al. 2014; Rai and Mishra 2017; Valson, Nazer, and Mukerjee 2017).

6. Complications of cesarean myomectomy

Among the most feared intraoperative and/or postoperative complication related to cesarean myomectomy, the massive hemorrhage represents a common condition investigated by researchers. In several studies, obstetrics tried to investigated the real incidence and relevance of intra and post-surgical hemorrage, referring to different variables as: absolute value of blood loss, point of hemoglobin lost, anemia, need of transfusion and number of units transfused, need of hysterectomy. Therefore, the heterogeneous meaning attributed to the concept of massive blood loss represented an interpretational limit in order to have a global view from available of literature. About that highly useful and interesting is the statistical meta-analysis conducted in 2017 by Pergiaolitis et al., who selected all observational studies on cesarean myomectomy published until then, which amounted to only nineteen, in order to highlight the most frequent and significant complications associated (Pergialiotis et al. 2017). They surprisingly reported that, although the 2,301 patients undergoing cesarean myomectomy compared to the cesarean delivery group, 1,599 women, had been exposed to a longer intraoperative duration and to a greater mean hemoglobin drop, this statistically relevant data did not necessarily translate into a greater and significant clinical risk for the patient. In fact, the mean difference of hemoglobin drop reported between the two groups only amounted to 0.25 mg/dL (95% CI 0.06–0.45) and on the other hand the mean difference of duration of the operation was 13.87 minutes (95% CI 4.78–22.95). It could be considered a reasonable unreached of surgical time if compared with the possible benefits deriving from the procedure, without any significant impact on the rapidity of postoperative patient recovery. Moreover, no difference on haemorrhage, transfusion rates, or postoperative fever was reported between the two groups and the prolonged hospitalization recorded in cesarean myomectomy group, though statistically significant, was minimum and scarcely relevant in clinical terms and for patient health all in all (Pergialiotis et al. 2017). This reconfirmed the safety and feasibility of the myomectomy during cesarean delivery already suggested by the findings of a previous meta-analysis conducted in 2013 by Song et al, who reported a longer surgical time and a greater hemoglobin drop, while not achieving statistical significance (Song et al. 2013). Two observational studies conducted in the same year of Pergiaolitis' meta-analysis (Rai and Mishra 2017; Valson, Nazer, and Mukerjee 2017) and further observational studies published in the following years, all retrospective and none prospective as mentioned above, have remarked the relatively safety of the procedure, not reporting a significant increase of blood loss and massive haemorrhage as intra e postoperative complication for caesarean myomectomy. Mostly, not evidenced a considerable worsening of clinical outcomes for this set of patients (Guler et al. 2020; Nargis, Karim, and Loverine 2019; Sparić et al. 2019, 2018; Senturk et al. 2017). As a matter of fact, a common finding is a very low rate of incidence of so-called major complications, as blood transfusion or hysterectomy, this latter almost null (Pergialiotis et al. 2017; Nargis, Karim, and Loverine 2019; Sparić et al. 2019, 2018; Senturk et al. 2017; Hatırnaz et al. 2020). For example Valson et al in their recent prospective study reported on a total of twelve women with fibroids underwent lower segment cesarean section with myomectomy following delivery only three cases, who needed blood transfusion after surgery. Although the several patient-myoma and procedure variables investigated, the authors did not find an exact reason to explain the onset of this complication (Valson, Nazer, and Mukerjee 2017). Instead Pergiaolitis et al, as mentioned above, on the basis of the analysed studies, detected an hemorrhage incidence, as a blood loss major than 1000mL, and rate of

blood transfusion as independent variables, which do not achieve a statistically significance p-value between the cesarean myomectomy group and the cesarean delivery group (Pergialiotis et al. 2017).

7. Blood loss during cesarean myomectomy: clinical strategy to contain hemoglobin drop

Despite its clinical relevance, the greater hemoglobin drop as in fact a constant findings in women underwent myomectomy removal after cesarean delivery, therefore a common trend, mostly in the recent studies, is to focus on possible strategies available during procedure in order to prevent and contain blood loss with the aim to test their real effectiveness. One of the most investigated is the pharmacological strategy, based on the employment of uterotonics, administrated in different ways and in different moments of the surgical procedure, favoring myometrium, especially the perilesional one, contraction in order to avoid atony and so massive bleeding. Already in 1999 Brown et al in his case-controlled study chose to resort to diluted oxytocin, which was directly injected in pseudocapsule of intramural and subserosal myomas of 16 women undergoing cesarean myomectomy and compared the results with a same sample cesarean delivery control group. Although the interesting idea proposed neither mean blood loss, hemoglobin levels and transfusion rate nor other surgical outcomes, as length of hospitalization and febrile illness were significantly different between the two groups (Brown et al. 1999). Later Ehigiegba et al on a sample of 25 pregnant women underwent cesarean myomectomy, experienced the use of intravenous oxytocin administrated in high dose immediately after delivery of the baby. They reported only five patients required blood transfusion and no one needed hysterectomy, therefore the authors concluded that high dose of oxytocin infusion intra and post-operatively could be a safe and wise choice Ehigiegba (Ehigiegba, Ande, and Ojobo 2001). On the contrary Dedes et al claimed that the use of uterotonics does not significantly modify the outcome following cesarean myomectomy rather than cesarean delivery alone, shifting the focus on other variables possibly related to peri-operative complications, as myoma and patients' characteristics (Dedes et al. 2017). In the more recent prospective study conducted by Rai and Mishra a possible solution used to prevent or minimize bleeding at myoma bed immediately after myoma removal during cesarean delivery is the local infiltration of dilute adrenaline, managing to achieving good results with a 20% of incidence of hemorrhage in the study group and no hysterectomy required at all. The data reported has been affected by the three cases of atonic peripartum hemorrhage in cesarean myomectomy group, treated with stepwise devascularization until adequate bleeding control (Rai and Mishra 2017). Considering this eventuality it is advisable to perform myomectomy always after baby extraction and afterbirth, even when the myoma is located anterior at the lower uterine segment, even if this let surgeon to opt for an unusual uterine incision as the longitudinal and/or corporal one, because in case of massive hemorrhage is fundamental to have clearly visible anatomical landmarks for proper and safe devascularization. About that several surgical techniques have been described recently for blood loss sparing during cesarean myomectomy. When a case on the basis of patient's and myoma's characteristics could be more exposed to massive blood loss is possible temporary block the uterine arteries bilaterally both using tourniquet and ligating or clamping them with a soft-ended instrument. Sapmaz et al published a randomized prospective study on a sample of 70 women in order to compare the effectiveness of the two methods mentioned above during cesarean section. They showed no statistically significant differences in term of perioperative blood loss between the use of tourniquet and the bilateral ascending uterine artery ligation, however it emerged that only this latter strategy ensured a good blood loss control even in the postoperative period, so that in the view of the authors it should be prefer (Sapmaz, Celik, and Altungul 2003). Instead Kwawukume did not evidence statistically proved benefit in the use of tourniquet tied both around the uterine arteries and ovarian vessels during cesarean myomectomy in comparison with cesarean alone in terms of blood sparing (Kwawukume

2002). Another possible choice is the electrocautery of myoma at the time of cesarean myomectomy as successfully experimented by Cobellis et al in 2002 on two cases of multiple fibroids of little or middle size (Cobellis et al. 2002). Moreover Incebyik et al. proposed the use of electro surgery associated to tourniquet and oxytocin infusion as bleeding prevent strategy for cesarean myomectomy. According to this approach first tourniquet is temporary applied at the cervico-istmic level, passing through the broad ligament, so electrocautery is employed for myoma removal and at the end of the uterine suture tourniquet is removed; postoperatively intravenous oxytocin is administered up to reach adequate uterine contraction. The results were encouraging and only two out of 16 patients required blood transfusion (Incebiyik et al. 2014).

8. Cesarean myomectomy outcomes

Assessing the intra and post-operative complication of cesarean myomectomy led many authors to explore the possible variables of each cases, which could affect the outcome of the procedure in order to have a clearly and wise evaluation of the case preoperatively, eventually guiding the choice to do or not myoma removal at the time of cesarean delivery. In these terms a really interesting study is the retrospective one conducted by Dedes et al in 2017, in which emerges that the significant increase of blood loss, meant as ≥500mL (p=0.02), rather that associated with the cesarean myomectomy procedure itself is related to large myoma size of ≥5cm, which, according to the results reported, is linked to this complication independent of a concomitant myomectomy or not (Dedes et al. 2017). This overturns at the same time both the idea to avoid cesarean section anyway and the idea of the absolute safety to not treat myoma during cesarean section. Maybe in this case removing myoma with a correct and wise technique could be the safer choice in spite of the risk? And if Zhao et al remarks the augmentation of risk of bleeding with myoma larger than 5cm maximum of diameter (Zhao et al. 2019), on the other hand Ehigiegba et al concluded that, employing the right technical precautions, even in case of big myoma cesarean myomectomy could be a safe option for the patient and for the surgeon (Ehigiegba, Ande, and Ojobo 2001). The question of the impact of the size of myoma on safety of cesarean myomectomy has been also well investigated by Kwon et al in their retrospective study of 2014, in which enrolled 65 patients underwent myomectomy versus 96 controls and, using the threshold of 5cm to differentiate the myoma labelled as large or not, did not find any statistically significant differences in consideration of the mean hemoglobin change, operative time and length of hospitalization (Kwon et al. 2014). Another ancient doubt is about the possible affection of the position of myoma on a greater incidence of bleeding, as a matter of fact in the past, while pedunculated or subserosal myoma removal was considered relatively safe during cesarean delivery, not the same point of view there was for the intramural lesions because of the feared relationship hypotized between the larger myometrium wound following removal and the incidence of atony and so massive blood loss. Actually, all the observational study up to nowadays do not report a statistical correlation between this complication and myoma location (Pergialiotis et al. 2017). On the other hand, is not well explored the real risk linked to a multiple myomectomy during cesarean section, mostly because almost the totality of the observational study excluded patients affected by more than one myoma, especially large and with different position, in order to prevent worst outcome. Dedes et al tried to dissipate this doubt and effectively found a greater incidence of massive blood loss in multiple cesarean myomectomies rather than the removal of single pedunculated or subserosal and intramural leiomyomas during cesarean section (OR respectively of 4.7 and 1.1.-1.4, 95% CI 0.8-26.3) (Dedes et al. 2017). The interpretation of these results could debatable in relation to the too much low threshold employed to define hemorrhage, 500mL, but to date are still one of the studies of reference on this question. None of the observational studies published evidence a significant relationship between the augmentation of intra and postoperative surgical and clinical risk and characteristics of the patient, with the exception of Dedes et al, who registered a significant postoperative drop in hemoglobin in women ≥40 years of age (OR 2.4 CI 95% 1.0–5.4, p=0.04) (Dedes et al. 2017). However, this

remains a controversial data which is not supported by other studies and so it could be considered not useful for practical implications.
Since the first attempt of cesarean myomectomy a technical constant, as reported above, is the need to postpone the myoma removing after baby extraction and placental expulsion not only for the anatomical reason already exposed, but also to ensure good neonatal outcomes, which in every study published are not affected in any way by the procedure, and at the same time to eventually manage first placenta related complications without getting worse maternal outcomes.

9. Surgical techniques to remove fibroids during cesarean section

On the other hand, contrary to Bonney's time, when gravid uterus used to be incised longitudinally (Jauniaux and Khan 2014), the recent attempts of cesarean myomectomy have been done in line with the more current and safer indications for cesarean section, according to which it should be preferable, when it is possible, a transverse incision of the inferior segment of the uterus to access to the uterine cavity for baby extraction. This consideration is fundamental for a preliminary evaluation, because the aim of the surgeon should be to try to limit the incision sites for myoma or myomas removing, using in all case is possible the incision just done. So that the myoma location predict the eventual necessity of further uterine incision over the extraction one and so, because of the number of the scars it could be needed, it could be a predictor of major risk of blood loss. Considering that anterior isthmic myomas could be the most eligible to cesarean myomectomy. Instead when additional incisions are needed different approach can be considered. In particular many of the different studies mentioned above do not the type of procedure employed to removal myoma and, when the incision of the uterine segment is not used, the conventional choice is a serosal myomectomy, which is performed through an incision over the site of myoma by the external face of the uterine wall, from which myoma is enucleated and then the dead space is closed. On the contrary recently a new and controversial technique has been proposed in a retrospective single institution study by Hatırnaz et al, the so called "endometrial myomectomy", which would be a less invasive and safer rather than the conventional fashion. As a matter of fact it is characterized by a small trans-endometrial incision at the site of myoma, performed after a quick and accurate visual and palpatory evaluation of location of the lesion, through which myoma is removed within the pseudocapsule; uterine breach is sutured only when the defect site is larger than 3cm with absorbable suture (Hatırnaz et al. 2020). The two main benefits of this procedure are: the absence of further scar on uterine surface and so the reduced risk of adhesion onset; the very small incision on the inner uterine surface which should be associated with a statistical significant lower intraoperative bleeding rather than serosal myomectomy (209mL versus 375mL, p=0.001) without at the same time the concrete remotely risk of intracavity adhesions or Ashermann syndrome (Hatırnaz et al. 2020). In spite of these considerations the endometrial approach has been criticized for several reason, like the possible affection of endometrial role in reproductive process (52) or the consequently higher risk of abnormal placentation (Sparić et al. 2017), but no evidences are available to date. Much more distinctive is the disagreement of Tinelli about the fibroid removal during cesarean myomectomy (Sparić et al. 2017), remarking the fundamental role of a "myoma pseudocapsule sparing" approach, described first by himself in 2014 (Tinelli et al. 2014). In fact, according to the biological mechanism that guide a correct wound healing in order to have better outcome especially in a so controversial procedure like cesarean myomectomy should be fundamental opting for the safest and optimal surgical strategy. Actually, scientific community has discovered the fundamental role of pseudocapsule in the management of fibroids, demonstrating that a preservation of the latter represents a way to preserve all the structure fundamental for a correct healing, like vessels, neurotransmitters, and neuropeptides, involved in inter-intracellular signalling. In order to correctly perform an intracapsular myoma removing during cesarean section according to Tinelli et al. it

is essential to do a sharp and exact dissection of the pseudocapsule and then to do an accurate approximation of the edges of the myometrium with a complete closure of all dead space (Tinelli et al. 2014). Applying this approach interesting really good outcomes has been registered in literature, in fact the 2014 prospective study conducted by Tinelli reported a mean hemoglobin decline lower in intracapsular cesarean myomectomy group rather than in cesarean delivery without myomectomy group (respectively 1.5 versus 1.6) (Tinelli et al. 2014), a result which is totally against the trend of the remaining observational study, with the only exception of Kwon et al (Kwon et al. 2014). Huang S.Y. et al fused the novel trans endometrial cesarean myomectomy technique proposed by Hatırnaz et al with the pseudocapsule sparing approach of Tinelli et al, demonstrating that it could be a safe treatment option without long-term adverse surgical outcomes and with better obstetric outcomes of subsequent pregnancy (Huang et al. 2018).

Another variable of cesarean myomectomy technique affecting clinical and surgical outcomes is the way chosen to obliterate the myometrium breach after myoma removal. As a matter of fact, mostly when an additional incision is needed to do or complete myomectomy, all the dead space have to be completely closed, otherwise that site can become full of blood and seat of hematoma with all the complications related. To date, we are not studies which investigated the best technique, instead each author reported several ways to closure according to the surgeon's experience. As can be seen from the various studies in the literature, fibroid bed can be sutured, beginning from the bottom, with one or more layers of interrupted Vicryl suture, generally choosing the size of needle on the basis of the myoma bed, up to a complete myometrial wall closure and with the aim to achieve haemostasis. For the superficial layer the surgeon can opt for continuous suture, introflexing or not, or simply for X stiches (36,39,43,44).(Sparić et al. 2018; Hatırnaz et al. 2020; Tinelli et al. 2014; Rai and Mishra 2017).

10. Conclusions

For a long time, it has been common and shared thinking that myoma should not be surgically treated in pregnant women at the time of birth through cesarean section in order to avoid complications for the patient. Nowadays the increasingly evidences reported in literature are gradually subvert the obstetrician point of view, leading to a concrete revaluation of this procedure.

Currently, the lack of randomized control trials make difficult to absolutely state the safety of the procedure and at the same time to definitively standardize the indications, but the several conclusions of all the observational studies available up to now surely give us elements to guide the choice to do cesarean myomectomy in specific set of women. Moreover, besides the clinical feasibility of the procedure itself, authors' experience and judgment agree with the evidences about the importance of the experience of the operator both to make safer the surgical procedure and to have intra-operative and after-surgery outcomes. However, the type technique should be preferred and then informative counselling and/or consensus complete of higher quality data than those currently available are topics that need standardization to make cesarean myomectomy safer for the patients and for the operator at the same time.

Therefore, we can conclude that a knowledge of all the aspects of this apparently controversial surgical procedure plus a consolidated surgical experience can give the instrument to offer an additional safe option of treatment, which to date cannot longer be avoided a priori, aiming to a tailored treatment for each patient.

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Training Teachers as Health Promoters

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Abstract

In the context of the global changes that affect families and communities, including the current COVID-19 pandemic, today's young people face new threats to their health and most of them lack adequate nutrition, healthcare services and education. As children and adolescents spend a significant amount of their time at school (or remotely connected with teachers, as occurred in these last months of health emergency), educators have the great opportunity to positively contribute to their global growth, fostering physical, mental and social wellbeing, which has also a significant impact on students' academic achievements. Nowadays, training teachers as "health promoters" allows educational system to deal more effectively with health needs of the students, helping them in the prevention of risky behaviours (cigarette smoking, binge drinking, drug use etc). According to the recommendations provided by the World Health Organization and UNESCO, we highlight the need for pre-service and in-service teacher training on the major topics concerning young people health, as well as COVID-19 safety issues. Moreover, teachers should be trained on the most participatory and pro-active methodologies to effectively convey health-related contents in school setting, in order to trigger a personal interiorization of the knowledge acquired by the students, and to engage them in practical actions about healthy lifestyles (i.e. balanced nutrition and physical exercise, no smoking, no alcohol, no drugs). Teacher training on health topics enables educators to develop a new professional identity based on a health-centered vision, in the perspective of reducing social/health inequalities that still concern most disadvantaged children. Medical professionals and pedagogists could be appointed as available consultants respectively for training teachers on health contents and about the most effective didactic strategies useful for displaying educational interventions in school setting aimed at preventing unhealthy habits among young people. Health education should be included in scholastic curricula within scientific disciplines or treated as separate subject in extracurricular activities under direct responsibility of school staff.

Keywords: Teachers Training; Health Promotion; Prevention; Risky Behaviours; School; Health Pedagogy.

1. Rationale: children and adolescents' facing new health threats

Each year 1.1 million of teenagers (10-19 years old) die due to preventable causes, and a huge part of them – especially those belonging to the most vulnerable social groups – start adopting risky behaviours (tobacco smoking, alcohol or drug use, unprotected sex, inter-personal violence etc.), which can lead to illnesses, injuries and premature deaths (World Health Organization - WHO 2018; Bauman 2011; Currie et al. 2009; Walker et al. 2011; Evans et al. 2013). Substance abuse during adolescence, as well as the acquisition of unhealthy eating habits since childhood (high intake of saturated fats, trans-fatty acids, free sugars with junk foods) represent health topics of major concern. Intentional self-harm, including "deadly selfies" and extreme experiences, are the most frequent cause of death among young people aged 15-29 years in Europe, while about 135,000 adolescents remain victims of

unintentional accidents that involve pedestrians, cyclists, cars, or motorcyclists (WHO 2018).
Heimlich manoeuvre, crossing streets and riding bikes are other important safety health issues to be addressed since early stages of life. Another impactful cause of accidental death is represented by drowning (up to 50,000 boys and girls dead per year) due to inadequate swimming skills or misperception of dangerous situations.
Concerning early pregnancies at global level, 44 births per 1000 belong to girls aged 15-19 (11% of overall births), and it is estimated that one girl out three (for a total of about 85 million young females) has experienced emotional, physical and/or sexual violence from husbands or partners. In less developed countries, infectious diseases, such as those preventable through vaccinations and free access to clean water and adequate hygiene (i.e. polio, measles, diarrhoea) still represent a big problem to be addressed. HIV-related deaths are decreasing worldwide with the exception of adolescents, who are largely affected from AIDS (2.1 million boys/girls in 2016, mainly concentrated in Africa) and often unaware of their condition, thus remaining usually untreated. Based on these epidemiological figures about infectious diseases worldwide, it is crucial providing young people with adequate information and knowledge in order to achieve the Sustainable Development Goal (SDG 3) set by the United Nations to eradicate AIDS, hepatitis, malaria, tuberculosis, and neglected tropical or water-borne diseases by the year 2030 (UNESCO 2015). Finally, half of the mental disorders resulting – at medium/long term – in depression, psychiatric conditions and suicide start by age 14, remaining largely undetected (WHO 2018).
Encouraging healthy habits since childhood is crucial for the prevention of risky behaviours and their harmful consequences in the future life and should be addressed by well-structured and multilevel programmes involving the entire society and all its stakeholders (Patton et al. 2016; Nutbeam 1997). Among those, schools - being the frontline educational institutions - can fulfil this critical and preventive task, giving the right information, providing support and a prompt feedback to students' needs, thus working for reducing health inequalities (Bakker and Mackenbach 2003; Nutbeam 2000). We are willing to highlight the need for pre-service and in-service teacher training on the major topics regarding young people health and on the most motivational methods to effectively convey these contents in school setting (Peterson et al. 2001).

2. Training teachers to handle students' socio-emotional and mental problems

In addition to physical health, an optimal emotional and social wellbeing enables individuals to cope with the challenges of life (WHO 2003; Sancassiani et al. 2015). The daily connection with children and adolescents gives schools, at least those who chose to display health educational interventions, the opportunity to impact students' global health during the critical ages of childhood and adolescence (Breslow 1999). Taking care of students' wellbeing has also undoubtable effects on their learning achievements, as documented in several studies (Zins et al. 2007; Gutman and Vorhaus 2012; West et al. 2004).
From primary to high school, teachers play a crucial role in the lives of young people, potentially able to spot early changes in behaviours that might represent signals of a mental discomfort (Russo and Boman 2007; Weare and Markham 2005). Unfortunately, the limited teacher education about health topics usually characterizes school systems, also in most developed countries (Fazel et al. 2014), and educators remain generally unprepared to deal with young people socio-emotional needs or mental problems, which can impair many aspects of students' life such as self-confidence and resilience, peer interactions, relations with educators as well as cognitive outcomes (Banerjee et al. 2016; Andrews et al. 2014). Even when educators are conscious of emotional difficulties experienced by students, they are unsure how to effectively support them or manage pre-critical situations. In other cases, the teachers are not able at all to recognize on time the early signals and identify students who need help, leaving them at risk of developing more serious problems (Reinke et al. 2011).
As a result, reactive responses, stigmatization or regulatory approaches are generally used rather than whole school approaches focused on prevention.
In the context of good educational policies, a "health promoting school" could generate a safe and supportive environment where teachers and learners address together (and possibly try to solve) health issues related to real experiences suffered by students. In some cases, to stop emotional problems from worsening complications, it would be enough just letting students talk with somebody available for a non-judgementally listening and understanding about the situations that children or teenagers are living (Moynihan et al. 2015; Young, 2005; Langford et al. 2014; Inchley et al., 2007). On the other hand, it has been computed that over 10 million students worldwide aged 13–18 require spe-

cialized assistance due to mental health conditions such as depression, anxiety, attention-deficit hyperactivity disorder (ADHD), and bipolar syndrome. About fifty percent of all mental illnesses begin before 14 years old, and 75% of those become evident by age 24 (Haggerty et al. 1994).

For various economic or social reasons, many adolescents with mental problems don't have access to professional help as early as possible. In this frame, well trained teachers might be able to identify mental illness at the onset and support students with the right tools (Ekornes et al. 2012). Mental problems impact dramatically students' academic performance and their interactions with schoolmates, as half of young people who experience a mental illness drop out of school (Vitaro et al. 1999). At the same time, teachers' inability to properly manage students with mental problems can make teachers themselves exhausted and burnt out, reducing their efficiency and sometimes causing early retirement. Responsibility for the mental health of the students should never be solely placed on teachers: a strong support system for educators is needed to reduce teachers turnover rate and to enhance their confidence dealing with these problematic situations (Ekornes 2017; Askell-Williams and Lawson 2013).

Of course, teachers cannot substitute health professionals (physicians, therapists, psychologists or family counsellors), but they should work together in order to better support the most vulnerable children and adolescents, establishing a continuous link between school and family environments. The key point is setting a whole-school approach, a caring and empathetic environment, where pupils feel safe to express their difficulties, without fear of being stigmatized (Michalos 2017). It is clear that teachers need to develop professional skills and knowledge on major young people health needs, as well as to acquire the best didactical practices, recognized as significantly effective in promoting students' wellbeing (Askell-Williams and Lawson 2013).

Ideal training programs are those aimed at preparing teachers to act as first aiders, capable to reassure and give information, raise students' self-confidence and refer those who need it to an appropriate professional support. Teachers equipped with the necessary knowledge, skills and tools might be able to detect in daily classroom routine pupils who experience a potentially critical issue and provide assistance without delay. This means responding to the adolescents socio-emotional difficulties before they result in a serious mental illness, in substance abuse or self-injuries (UNICEF 2002). In the contemporary social context, educators must be able to assess the risk of suicide, understand the early signs and risk factors of major mental illnesses, depressive syndromes and addictions.

3. How to train teachers as health promoters

Teachers perceive themselves as having the main responsibility for implementing health educational interventions at school, but they simultaneously face a general lack of training to adequately promote students' physical, mental and social wellbeing (Jourdan 2016). Scientific evidence makes it clear that there is a strong need for school-based strategies in health education area in the frame of a "health promoting school approach", where every experience and activity in the classroom is regarded as an opportunity to improve students' global development. At the same time, it is challenging for the teachers to address health topics in everyday school routine without a specific training. Currently, teachers are not always sufficiently provided with additional pre-service or professional learning opportunities to assist them in developing student health (Byrne et al. 2015; Leger et al. 2007). Specific investments in strengthening the capabilities of teachers to implement health promotion in schools are still needed, as we are far away from reaching this goal.

The teachers training should be able to trigger cognitive and behavioral processes and provide educators with a wide range of methods and tools for organizing, managing and optimizing their teaching health-related practices in the classroom.

Twelve specific competences in health education (combining knowledge, attitudes and skills) have been identified on a small sample of researchers from nine EU countries, Canada and Australia – with expertise in this field – belonging to the Schools for Health in Europe (SHE) Network and the International School Health Network (ISHN), two big international organisations aimed at promoting school-based health education (Moynihan et al. 2015). Starting from a list of 36 items, the following twelve competences were selected by the experts involved by Moynihan and colleagues: knowledge health determinants; communication skills; ability of the teacher to act as a "researcher"; pedagogical health content knowledge; general content knowledge of health issues; fundamentals of general pedagogy; knowledge of health education/promotion theories and models; skills in planning, implementing and assessing whole-school health promoting initiatives; knowledge of health education curricula; knowledge of learners and their characteristics; ethical skills; willingness to engage in whole-school and community health promoting activities (Moynihan et al. 2015).

Pre-service teachers' health education should last several years, while in-service training can be organised on regular basis (every year) for groups of teachers coming from the same or different schools (Moynihan and Mannix-McNamara 2014) and be placed at the heart of any teachers' educational path. A successful teachers' training program (both pre-service and in-service) about health-related topics must not be limited to sporadic or fragmented information sessions that leave gaps in knowledge, but it should be implemented on a long-term period.

Aims, principles and the different parts of the training should be explained at the beginning of every session. Training sessions should be flexible, combine various formats parametrized on learners' demands and institutional needs, targeted at development of required competences; at the same time, the intervention should give the opportunity to apply the new skills acquired, recognizing and developing personal interests and points of strength and able to motivate teachers in taking action.

The efficacy of training programs seems to increase when experiential workshops and interactive educational sessions with health professionals are used, having teachers directly involved in the participatory learning methods (Dewey 1986; Ahmed et al. 2006). Indeed, health contents should be re-constructed by the teachers in order to be successfully transferred to their students (Bandura 2001). Pre-training evaluations and post-training follow-up assessments could be performed (also in a subsequent operational phase, when trained teachers display their activities with students in the classroom) to monitor the outcomes of these specific teacher educational programs (Kutcher et al. 2013).

The socio-emotional competences (SEC) of the teachers are also important factors to be taken into account during pre-service training, as they are crucial for the development and maintenance of a supportive teacher-student relationships and an effective classroom management. A systematic review of twenty studies has demonstrated that teachers' health education increases their self-efficacy to motivate students about health choices and to support children already suffering from unhealthy behaviours (Shepherd et al. 2016). Another review underlines the importance of teachers' physical and mental wellness, required to nurture students and contribute at fully developing their potential (Pillay et al. 2005). The personal health attitudes and behaviours of teachers are crucial factors for successfully impacting their students in terms of both learning outcomes and wellbeing promotion (Yager 2011). In other words, teachers need to "feel safe" on the frontlines when they start a change process that involves day-to-day routine with students (Pickett et al. 2015).

Finally, a comprehensive teacher training health program should focus also on the educational use of technological tools and facilities, which can help to better address students with special educational needs and reduce health inequalities among vulnerable schoolchildren. Smartphone apps, audio-visual stories or educational games can increase students' motivation to carry on with the acquisition of health-related contents and stimulating them to maintain the newly acquired correct lifestyles, improving students' self-management at long term (Oomen-Early and Early 2015).

External experts (coming from University, Professional Associations or Healthcare Services) may provide effective training in teachers' health education and remain available for ongoing consultation for students' who need professional support that cannot be guaranteed by educators.

Teachers are recommended to involve all the school community members, cooperate and make networking with colleagues at global level (attending international seminars, webinars, meetings) to update their qualification programmes according to global standards (Hung et al., 2014; Flaschberger et al. 2012). Health education interventions are most effective if parents are involved: families can complement and reinforce at home what children learn at school. In this perspective, it is useful to ensure a constant consultation between parents and teachers and establishing consistency of approaches between them (Murray et al. 2013; IUHPE 2008).

However, every organization, including school system, has to cope with the low propensity of its staff to make full use of all the newly available training opportunities and to modify their educational practices by accepting to work with new methods (Byrne et al. 2018). Unwillingness could be due to the way in which teachers view their role and its social recognition, thus claiming for public awareness of the relevant position that educators have in the society (Charlton 1981).

4. Teacher training on active methodologies to convey health related contents

School is the ideal setting to provide basic health information and display related educational interventions (Hung et al. 2014). In this perspective, teachers should be adequately trained to increase young generations' access to a functional, interactive and critical health literacy, and their ability to use it

effectively in the real life. Pedagogic consultants could equip teachers with the pro-active didactic strategies - going beyond the simple transmission of information – needed to raise students' motivation towards healthy habits and stimulate a personal re-construction of knowledge along with a critical thinking about harmful consequences of risky behaviours (Bandura, 2004),

Moreover, teachers should be trained on how to incorporate a wide range of motivational activities in the repertoire of their teaching skills as useful instruments for sharing health knowledge, values, and behaviours in a transversal and interdisciplinary way. Engaging children and adolescents in practical actions about healthy habits could trigger a deep and transformative learning, capable to enhance individual capacity to protect their own health lifelong (Dewey 1986; Mezirow 1997; Lengrand 1989; Rowe 2008; Michel et al. 2009).

In order to encourage young people to take more control of the learning process and environment, as well as to strengthen transversal life skills, a huge number of activities can be carried out in school daily practice: class discussions, small working groups, brainstorming, role play, tasks of reality, educational games and simulations, debates, audio and visual activities, case studies, storytelling, theatre, dance/music. These activities, along with promotional methodologies can enhance all the different talents and ways of learning of the students, and allow teachers to build up a friendly and supportive learning space, that encourages students to freely express their emotional difficulties without feeling "under judgement" (Payton et al., 2000). This kind of cooperative and non-competitive environment is also the most impactful way to cope with bullying behaviours and opponent or aggressive attitudes, strengthening students' resilience and self-efficacy (Wilson and Lipsey, 2007; Seligman et al. 2009; Elias et al. 1994). In this frame, teachers act as facilitators who listen to the students' health questions, and provides them with useful suggestions, opportunities and new perspectives within a democratic, participatory school community, rather than adopting reactive and regulatory approaches (Jennings and Greenberg. 2009).

5. Conclusions

Students' health promotion represents an intrinsic ethical duty of scholastic institutions, calling for an adequate pre-service and in-service teachers' training both on the main health issues related to young people and the most effective methods to deliver health knowledge in daily classroom activities.

Training teachers as "health promoters" represents a possible response to the new global challenges in the field of education and healthcare (Jourdan et al. 2008). Well-trained teachers with a new professional identity and a health-centered vision can make a difference in the prevention of harmful behaviours (tobacco smoking, alcohol and drug use, screen-related or gambling addictions, betting online, consumption of junk food) among young people (WHO, Regional Office for Europe 2018; He et al., 2010; Goran et al. 1999). Medical professionals and pedagogists could be respectively appointed as consultants for training teachers on health contents and about the most effective didactic strategies for displaying educational interventions aimed at promoting students' wellbeing in school setting. Health education should be included in scholastic curricula within scientific disciplines or treated as separate subject in extracurricular activities (Pearson et al. 2015; Kilgour et al. 2015; Paakkari and Paakkari 2012). Teachers should act as first aiders and promoters of young people global wellbeing, thus impacting not only students' behaviours, but also their families and communities (Murray-Harvey and Slee 2007). The success of health educational interventions is directly related to the effectiveness of teachers' training on the major health topics and on the pedagogical practices for working with students on these specific issues.

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