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Italian Journal of Gynaecology & Obstetrics

Management of obstetrics and gynaecological patients with COVID-19

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ABSTRACT

The widespread SARS-CoV-2 implies the application of procedures aimed to detect, isolate, and appropriately manage affected patients in the setting of obstetrics and gynaecologic emergency room and in inpatient setting, such as during labour, delivery, and postpartum. Here we report specific recommendations for the management of suspected and confirmed gynaecologic and obstetrics patients with COVID-19. The checklist developed by the Società Italiana di Malattie Infettive e Tropicali (SIMIT-2, available in English, Italian, Chinese) represents the first step to classify patients who need to be managed following the SIMIT-1 flowchart, applying all the appropriate infection control procedures. In this scenario, the management of pregnant women needs to follow the same procedures as the general population. Nevertheless, as for other potentially severe respiratory infections, pregnant women could be more vulnerable. In this regard, the maternal and foetal interests can be conflicting, such as the choice of the time and mode of delivery or the use of steroids for foetal maturation. Moreover, available evidence suggests a maternal-foetal transmission via contact with respiratory secretions and seems to exclude in utero transmission. Therefore, the appropriate management of breastfeeding is unclear, and the temporary separation of the infant from the mother could be an option. Finally, in general, delivery represents a moment of a high risk of infection for healthcare providers, and specific behaviours are mandatory.

SOMMARIO

L'ampia diffusione del SARS-CoV-2 rende mandatorie l'applicazione di procedure volte a rilevare, isolare e gestire i pazienti affetti, sia nel pronto soccorso ostetrico-ginecologico sia in regime di degenza, come durante il travaglio, il parto e il postpartum. Per tale ragione, qui riportiamo raccomandazioni per la gestione in ostetricia e ginecologia di casi sospetti o confermati di COVID-19. La checklist sviluppata dalla Società Italiana di Malattie Infettive e Tropicali (SIMIT-2, disponibile in inglese, italiano, cinese) rappresenta il primo passo per classificare i pazienti che devono essere gestiti seguendo lo schema SIMIT-1 e applicando tutte le procedure necessarie per il controllo delle infezioni. In questo scenario, la gestione della donna in gravidanza deve seguire le stesse procedure della popolazione generale. Tuttavia, come per altre infezioni respiratorie potenzialmente gravi, le donne in gravidanza potrebbero essere più vulnerabili. Di conseguenza, gli interessi materni e fetali possono contrastare, come la scelta del momento e della modalità del parto o l'uso degli steroidi per profilassi della prematurità fetale. Inoltre, i dati disponibili sembrano escludere la trasmissione intrauterina del SARS-CoV-2 suggerendo invece la possibilità di un'infezione post-partum. Pertanto, la gestione appropriata dell'allattamento al seno è incerta, e la separazione temporanea del bambino dalla madre potrebbe essere necessaria. Infine, in generale, essendo il parto un momento ad elevato rischio di infezione per gli operatori sanitari, comportamenti specifici sono obbligatori.

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INTRODUCTION

In December 2019, a novel coronavirus was identified as the cause of some pneumonia cases in Wuhan, a city in the Hubei Province of China (1). In the following weeks, the infection rapidly spread across China and other countries around the world.(2) On February 12th, the World Health Organization (WHO) designated the disease as COVID-19 (Coronavirus Disease 2019) (3).

Coronaviruses are an important cause of the common cold, probably second only to rhinoviruses in frequency (4). Nevertheless, in 2002 and 2012 two different coronaviruses causing severe respiratory illness in humans emerged (SARS-CoV and MERS-CoV), and this new recently isolated virus has 79% nucleotide identity to SARS-CoV and about 50% to MERS-CoV.(4) The genomic sequence of the new virus has been early identified with laboratory confirmation achieved by the Chinese Centre for Disease Prevention and Control (CDC) before January 23rd. Based on the phylogenetic similarity with SARS-CoV, the Coronavirus Study Group of the International Committee on Taxonomy of Viruses proposed the name Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) to designate the 2019-nCoV virus (5).

Bats seem to be the natural reservoir of both SARS-CoV and MERS-CoV, and the phylogenetic analysis shows consistently data with a bat reservoir for also the SARS-CoV-2. Noteworthy, it seems that another animal played the role of intermediate host between bats and humans (4). However, human-to-human transmission has been confirmed in China (6) and is thought to occur mainly via respiratory droplets (7), with a preliminary estimate of the median incubation period of 5-6 days (ranging from 0-14 days) (8). Preliminary shedding studies have shown that the transmission can occur during the early phase of the disease in asymptomatic patients, contributing to the overall diffusion (2,9). This impacts dramatically on the effectiveness of screening of suspected cases and prevention measures.

Different studies found that clinical characteristics of COVID-19 mimic those of SARS, although there are some clinical aspects that differentiate COVID-19 from other respiratory infections, such as SARS, MERS, and seasonal flue. Clinical

symptoms at presentation are not specific and the disease usually presents with respiratory symptoms such as fever, cough, and dyspnea (2,9). Pneumonia seems to be the most frequent serious manifestation of infection, and it presents with bilateral infiltrates on chest imaging (1). From a Chinese report of 44,500 confirmed cases, 81% of them were mild, 14% were severe, and 5% critical (11). The WHO on February 19th gave some data on the case fatality rate (CFR). Although the CFR for COVID-19 has been reported significantly lower than for SARS and MERS, it was estimated ranging between the 1.4% and 2.1% versus the 9.6% and 40% for SARS and MERS, respectively (10). Within China, the confirmed CFR, as reported by the Chinese Centre for Disease Control and Prevention, is 2.3%, with a risk of serious illness that rises with age and with the presence of comorbidities (1). In the same WHO report published on February 19th, data based on the estimated number of total infections calculated through modelling suggest an overall Infection Fatality Rate (IFR) ranging from 0.3% to 1% (12). Although antiviral agents are under evaluation for efficacy in COVID-19 such as remdesivir and lopinavir/ritonavir, the clinical impact is still unknown and further studies are needed for verification (13,14). Conversely, recent evidence suggests a possible application of chloroquine and hydroxychloroquine as a molecule able to reduce the exacerbation of pneumonia, duration of symptoms, and delay of viral clearance, with limited severe side effects, although further evidence is required (15).

Regardless of adopted preventive measures, the number of cases is growing globally. On March 8th the total confirmed cases were 105,586, with 80,859 cases confirmed in China and 24,727 cases confirmed outside China and a total of 101 countries that have now to face this new virus. On March 11th, the WHO made the assessment that COVID-19 can be characterized as a pandemic (8).

In Italy, the first two cases were isolated at the end of January, and on March 8th, 5883 cases with 234 deaths have been reported. Noteworthy, reported numbers are likely underestimates since milder cases are less likely to be reported and tested to identify the SARS-CoV-2 by polymerase chain reaction performed on specimens collected from the upper respiratory airways

(nasopharyngeal and oropharyngeal swab).

A review has been published recently giving numbers on R0 for COVID-19. It seems that the speed of the spread is much faster than that reported for SARS (16). So, even if the CFR for COVID-19 is far lower than that for SARS the high transmissibility could eventually result in more severe cases and deaths (17).

In this scenario of a widespread infection, only the application of public health interventions, such as early case isolation, some forms of mobility restrictions, social distancing, and behavioural changes at the population level can be effective in controlling the spread. Additionally, there is emerging evidence that nosocomial transmission plays a major role in transmission, accounting for infection of 29% of affected healthcare providers (HCP) and 12% of hospitalized patients (18). In this regard, obstetrics and gynaecologic departments have to cope with a consistent flow of patients presenting every day at the Accident and Emergency (A&E) unit and in the delivery room. The presence of a procedure with the goal of prompt detection and effective triage and isolation of potentially infectious patients is essential to prevent exposure among patients, HCP and visitors. Moreover, obstetricians and gynaecologists have to consider how to manage pregnant women and infants in the case of suspected infection, particularly in the delivery room and during breastfeeding. On that basis, the presence of a multidisciplinary team responsible for implementing procedures to face this new situation is of paramount importance as well as the development of specific protocols and recommendations, such as those here reported that has been accepted for the management of suspected and confirmed COVID-19 cases at our institutions.

MATERIALS AND METHODS

A thorough consultation of medical literature and of public health authorities and scientific societies guidance documents was performed. A multidisciplinary team composed of the heads of Obstetrics and Gynaecologic, Paediatrics, Infectious Diseases, Intensive Care Unit, and Public Health Departments discussed and developed the recommendations.

RESULTS

Here we report the procedures and recommendations collegially discussed and approved for the management of women presenting to an obstetric triage unit or admitted to maternity ward.

General approach (Figure 1):

1. Any woman visiting the A&E department must be screened for the presence of symptoms and epidemiologic risk factors with the checklist developed by the Società Italiana di Malattie Infettive e Tropicali (SIMIT-2) (available in English, Italian, Chinese at (<http://www.simit.org/IT/index.xhtml>)).
2. The Flowchart reported in SIMIT-1 card must be applied to each patient (<http://www.simit.org/IT/index.xhtml>).
3. Early recognition of COVID-19 suspect cases at the triage entrance is vital to immediately implement infection control procedures. This is particularly true for women presenting with an obstetric urgency-emergency (19).
4. Any case has to be classified in one of three main groups in order to identify suspect cases.

Group 1: Asymptomatic patient AND epidemiologic risk factors.

Epidemiologic risk factors are defined as at least one of the following during the 14 days prior to symptom onset:

- History of travel to or residence in China during the 14 days prior to symptom onset.
- History of travel or frequentation of a "red zone" (high prevalence setting according to national indications).
- Close contact with a confirmed or probable case of SARS-CoV-2 infection.
- Worked in or attended a health care facility where patients with confirmed or probable SARS-CoV-2 acute respiratory disease patients were being treated.

These women have no indications for admission, and there is no indication to perform a

nasopharyngeal swab to asymptomatic patients. Clinicians must stress the importance of:

- Checking body temperature every day and paying attention to the possible onset of symptoms. In the case of symptoms, patients must contact the Health Services to ask for a consultation and to receive specific instructions. A specific phone contact must be provided.
- Notifying the case sending an email to the Hygiene and Public Health Service.

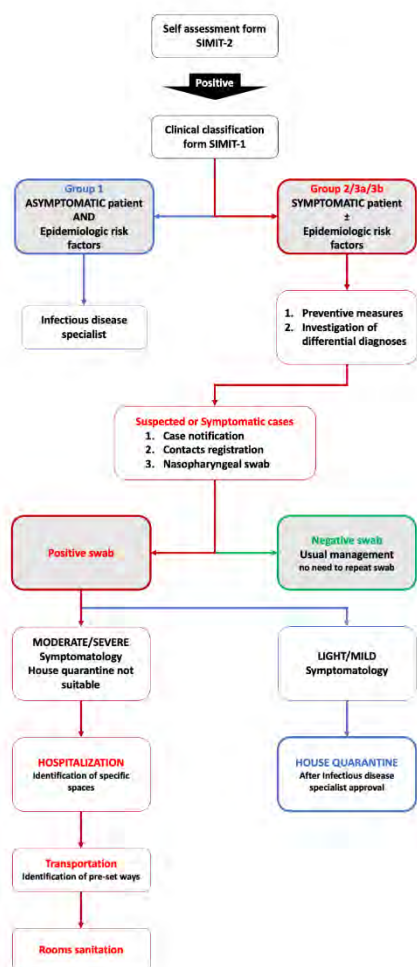


Figure 1. Flow chart for the general management of a patient referring to the obstetrics and gynaecologic department.

Group 2: Symptomatic patient AND epidemiologic risk factors.

These women represent suspect cases. Patient with a severe acute respiratory infection

(fever of any degree, cough, and dyspnoea) AND with no other aetiology that fully explains the clinical presentation AND at least one of the following during the 14 days prior to symptom onset:

- History of travel to or residence in China during the 14 days prior to symptom onset.
- History of travel or frequentation of a “red zone” (high prevalence setting according to national indications).
- Close contact with a confirmed or probable case of SARS-CoV-2 infection.
- Worked in or attended a health care facility where patients with confirmed or probable SARS-CoV-2 acute respiratory disease patients were being treated.

Definition of close contact includes a person involved in any of the following situations that must have taken place 14 days before or after the beginning of symptoms in the confirmed COVID-19 case:

- HCP or people providing direct care for SARS-CoV-2 patients who have NOT USED personal protective equipment (PPE) and laboratory personnel who has NOT USED personal protective equipment during specimen handling.
- Working or sharing the same closed environment with SARS-CoV-2 patients.
- Living in the same household as a SARS-CoV-2 patient within a 14-day period after the onset of symptoms in the case under consideration.
- Aircraft passengers who were seated in the same row as the case, or in the two rows in front or two rows behind a confirmed COVID-19 case, trips close contacts, crew members.

Group 3a: Symptomatic patient WITHOUT epidemiologic risk factors.

If a patient presents with fever > 37.5°C and cough, even if without a clear epidemiologic risk factor, it will be managed as a suspect case.

Group 3b: Inpatient women with onset of symptoms during the hospital stay WITHOUT epidemiologic risk factors.

If a woman develops fever $> 37.5^{\circ}\text{C}$ and respiratory symptoms while inpatients the case must be managed as a suspect case.

The clinical suspect must rise only in women with no other possible symptoms cause, and isolation must be respected until the final swab result.

5. Infection control procedures.

Once the suspect case is identified, the infection control procedures must be immediately implemented in order to prevent SARS-CoV-2 diffusion. HCP should immediately adhere to Standard Contact and Droplets Precautions upon patient arrival and during visit.

I. The suspect case:

- Must wear a surgical mask.
- Must be isolated in a single room at a negative pressure with a minimum of 6 air changes per hour (if not available the patient can be isolated in a room with adequate air changes).
- It must be kept at least 2 meters during the interview.

II. The health care providers:

- Must wear a facemask, favouring FFP2 based on local situational analysis of supplies (FFP2 or FFP3 facemask are recommended if handling airways generating aerosol such as nasopharyngeal swab, intubation, bronchoscopy, broncho aspiration, etc.)
- Must wear a non-sterile gown.
- Must wear two pairs of gloves.
- Must wear eye protection.
- Must perform correct and scrupulous hand hygiene.
- Must implement staff cohorting (the suspect case should be assisted by the same team of HCP).

III. The number of contacts must be reduced to a minimum. No visitors are allowed.

IV. All non-dedicated, non-disposable medical equipment used for patient care should be cleaned and disinfected.

V. Even in the case of an obstetric urgency/emergency the staff should firstly

implement infection control procedures as far as possible (19).

Regarding the infection control procedures by HCP during the interaction with suspect/confirmed cases, the standard surgical-style mask can able to prevent both the acquisition and the transmission of SARS-CoV-2, limiting the recommended use of FFP2 or FFP3 facemask by HCP during procedures generating aerosol.(20) These recommendations are based on evidence supporting the droplet transmission as the main transmission route of the SARS-CoV-2, similar to other respiratory viruses such as influenza (20), with controversies about the role of airborne transmission route (21).

Conversely, the prophylactic continuous use of the surgical-style mask to prevent the transmission of respiratory disease is supported by more limited evidence.(22) This because a comprehensive and appropriate application of all the infection control procedures is required to appropriately limit the transmission (22). However, prophylactic continuous use of the surgical-style mask, at least by HCP, can be considered an appropriate recommendation based on local situational analysis of supplies and prevalence of patients affected by COVID-19.

The actual proportion of asymptomatic patients affected by SARS-CoV-2 is still undefined, with evidence supporting asymptomatic patients as a possible source of infection with viral load comparable to symptomatic patients (9,20,23) Moreover, the prophylactic use of the surgical-style mask might improve the perception of safety, reducing stress and frustration among HCP, and may help to improve adherence to other infection control procedures acting as a physical barrier to prevent touching the face (24).

On that basis, the prophylactic use of the surgical-style mask by HCP can be recommended to prevent the acquisition of SARS-CoV-2 from any patient regardless of symptoms, as well as prevent the transmission of SARS-CoV-2, being any asymptomatic HCP a potential source of infection (9,20,23). Similarly, prophylactic use of the surgical-style mask by all patients could be recommended (25).

6. Notification and diagnosis.

Guidance from local health authorities for the definition of suspect case must be followed. Whenever possible, an infectious disease

specialist should be involved in the decision whether a patient has signs and symptoms compatible with COVID-19 and whether the patient should be tested.

In any suspect case, three steps must be implemented:

I. Immediate notification of the case.

II. Immediate implementation of contact tracing measures:

- All the people entering the room must be registered. They will have to monitor their signs or symptoms for the following 14 days.
- Close contacts must be identified and must be informed that they will have to undergo active monitoring, including restriction from work in any healthcare setting until 14 days after their last exposure. In the case of symptoms, they should immediately notify and self-isolate.

III. Collection of the diagnostic respiratory specimens (nasopharyngeal swab).

- This specimen must be collected using the PPE by the person who provides care to the patient and in the same room where the patient is isolated.
- The specimen can be stored at 4°C for up to 72 hours.
- The notification form must be sent to the laboratory along with the specimen.
- Given varying differential diagnosis in people presenting respiratory symptoms, testing for other respiratory pathogens is encouraged.

7. Nasopharyngeal swab interpretation.

From the collection of the diagnostic respiratory specimens until the result, the patients must be monitored in isolation.

I. Negative result: With a negative result, there is no indication to swab repetition, and the patient can be discharged with medical charges exemption 5G1.

II. Positive result: If the test results positive, the infection is confirmed.

In Italy, at the moment, the final confirmatory test is performed at the National Institute for Infectious Diseases Spallanzani, Rome. The suspect case can be confirmed only after this official confirmation. Repetition of sampling is needed to confirm microbiological recovery. In general, a patient can be considered negative only after 2 consecutive negative results within 24 hours. The current indication is to repeat a second sample, in patients with resolution of symptoms, after 7 days from the first positive results.

8. Choice of inpatient or outpatient management.

I. Outpatient management:

- After consultation with the Infectious Diseases Specialist, a patient confirmed with COVID-19 but with mild or moderate symptoms (and no other Obstetric or Gynaecological indication for admission) can be considered for home care if the residential setting is suitable.
- The patient must inform its general practitioner about its clinical situation by telephone call.
- The Hygiene and Public Health service must be informed about this choice.
- Patients can be discharged with medical charges exemption 5G1.
- The woman is advised to go immediately home by her own personal transport, to self-isolate, and to wait for specific indications from the Hygiene and Public Health service. Any appointment will be rebooked in 14 days (19).

The following recommendation has to be provided for the home care of the patients with suspected COVID-19:

- A single separate room, well ventilated, must be reserved for the patient.
- The number of visitors must be reduced.
- Family members must use different rooms without sharing spaces.
- A dedicated bathroom with windows must be used if possible.
- Caregivers should use precautions while looking after the patient.

- Thorough hand hygiene must be respected.

Based on the gestational age, a daily phone follow-up to monitor obstetric symptoms, such as fetal movements, vaginal discharges, uterine contractions, should be considered in pregnant women with COVID-19.

In case of emergency, the pregnant woman will be transferred to the hospital calling the emergency number (118), with neither husband nor another trustworthy person should accompany her. Before arriving at the hospital, the triage nurse should be informed, calling the number provided to the patient in order to allow HCP to get organized.

II. Inpatient management:

Patients critically ill must be immediately transferred to an Intensive Care Unit setting for adequate support. Patients with mild or moderate COVID-19 symptoms, but with an Obstetric or Gynaecological indication for admission should be managed as follows:

- Confirmed cases must be transported from the room of first evaluation to the final designed room using short and predefined routes, always respecting precautions.
- If elevators are used to move infected patients these must be sanitized immediately after the use.
- Appropriate rooms must be identified for the isolation of the patient in the Obstetric ward, Gynaecological ward, and Delivery Room.
- Staff providing patient care should be the same during each shift.
- If a surgical operation is necessary for obstetric or gynaecologic reasons precautions as for other infective diseases should be adopted. It is paramount to alert and inform the anaesthesiologist given its high exposure risk during airways management. In operation scheduling, it would be appropriate to perform the surgical intervention last in the operative list.

9. Environmental Cleaning and Disinfection.

Routine cleaning and disinfection procedures are important for SARS-CoV-2 in healthcare settings. A cleaning service must be available for this procedure. Noteworthy, after the usual obstetric and gynaecologic evaluation of suspect or confirmed COVID-19 patient, ultrasound transducers should be cleaned and disinfected based on the manufacturer specifications as well as other surfaces.

Specific situations: Pregnancy, labour, and delivery.

1. General management of pregnant women.

In the absence of specific vaccine or treatment, the only available public health tools to control person-to-person transmission are isolation and quarantine, social distancing and community containment measures (26). Moreover, considered the fact that nosocomial transmission plays an important role in diffusion, it is important to try to reduce as much as possible the attendance for routine/non-urgent antenatal care in women with suspected or confirmed COVID-19. This could be done with woman cooperation. Routine appointments for women with suspected or confirmed COVID-19 should be rearranged until the end of the recommended period of isolation. More urgent appointment rearrangements will need the discussion with a senior obstetrician to balance risks and benefits.

The same can be said for planned inductions of labour or planned caesarean sections in women with suspected or confirmed COVID-19. An individual assessment should be made to determine whether it is safe to delay the appointment with the aim to minimize the risk of infectious transmission to other women, HCP and, postnatally, to her infant.(19) If obstetric care cannot be delayed all precautions should be adopted in order to reduce transmission.

2. Management of pregnant women with COVID-19.

Much is unknown about COVID-19 in pregnancy with less than 20 reported cases.(27,28) The management of pregnant women with COVID-19 (suspected or confirmed) should be similar to the management of non-pregnant

women, with the consideration that pregnant women, as for other potentially severe respiratory infections, such as influenza, SARS, or MERS, could be more vulnerable to developing severe sequelae. Data on illness associated with other virulent coronaviruses can provide insight into COVID-19 effects during pregnancy.(29) However, given the limited available evidence on COVID-19 in pregnancy,(27,28) it is not clear if pregnant women with COVID-19 will suffer from a more severe disease.(29) In consideration of the possible impact of the disease on pregnant women, The Clinical Practice Guidelines no. 225(30) for SARS during pregnancy stressed the importance of informing the woman about:

- Possible effects of SARS-CoV viremia on the foetus.
- Possible foetal risks caused by maternal respiratory failure.
- Option of termination of pregnancy in the event of severe maternal compromise up to 22 weeks of gestations (originally 24 weeks).
- Obstetrical management between 24 and 34 gestational weeks or after 34 gestational weeks, including discussion about the mode of delivery, type of anaesthesia, possible use of antibiotics and corticosteroids (betamethasone), possible preterm delivery in the case of severe maternal impairment, and possible perimortem caesarean delivery in the case of fatal maternal complications.

After the admission of a suspect or confirmed case of COVID-19 in a pregnant woman, a multidisciplinary meeting should be set in order to plan the management. The woman should be informed about the conclusions, and a discussion with her should be conducted (19). Available literature addressing the topic of COVID-19 management in pregnancy reports the following general principles regarding the management of pregnant women with confirmed or suspected COVID-19 (29):

- Early isolation and implementation of infection control procedures.
- Careful evaluation of specific needs, such as oxygen therapy, antibiotic therapy to prevent bacterial secondary

infections, and early mechanical ventilation in the case of respiratory failure.

- Close monitoring of maternal-foetal wellbeing:
 - a) Maternal observation with vital signs monitoring. If a woman develops signs of sepsis, it is important to consider the wide range of possible differential diagnosis and investigate the possible aetiology, following the protocol for sepsis in pregnancy.
 - b) Given the high rate of foetal compromise observed in the reports of cases with COVID-19 in pregnancy (27,28), a pregnant woman with COVID-19 should have her foetuses monitored with continuous electronic monitoring during labour (19).

Moreover, specific aspects to take into account in a pregnant woman affected with COVID-9 are:

- a) Changes on foetal heart rate patterns can be an early sign of maternal respiratory deterioration.
- b) The target of blood oxygen saturation in spontaneous breathing is 92-95% when oxygen therapy is needed for severe respiratory conditions (31).
- c) WHO advises against the use of corticosteroids on clinical management of severe acute respiratory infection unless indicated for another reason (32,33).
However, given the benefits of betamethasone for foetal lung maturation, and the lack of evidence of harm in women with COVID-19, this therapy should be administered when indicated (19).
- d) Given the wide differential diagnosis in people presenting with acute respiratory symptoms, the Infectious Disease Specialist could decide to start an antiviral or antibiotic therapy, especially before test results for COVID-19 (31).
- e) At the moment, some antiviral agents are under evaluation for the treatment of COVID-19 but no data are available in pregnancy. Remdesivir is being studied in a randomized controlled trial in patients with SARS-CoV-2 and it has been used in one case in the USA. Nothing is

known on the passage of this drug into breastmilk, but data from a patient breastfeeding with Ebola shows no adverse effect in the infant (34).

- f) Imaging investigations (such as chest X ray and computed tomography) should be performed as for non-pregnant women, implementing all the measures to protect the foetuses from radiations exposure (19).
- g) If maternal stabilisation is needed, this has to be considered a priority before delivery, as in other maternal emergencies (19).

3. Management of labour and time of delivery in patients with confirmed or suspected COVID-19.

The Clinical Practice Guidelines for SARS during labour and delivery could be considered for a patient with COVID-19 (30).

- A multidisciplinary team consisting of Obstetrician, Nurses, Paediatricians, Infection Control Specialist, Anaesthesiologist should be identified in each unit and be responsible for the organization and implementation of management protocols.
- Preventive measures should always be respected while taking care of women with confirmed COVID-19 or under investigation. The use of FFP2 or FFP3 face mask is recommended at least during the second and third stages of labour based on local situational analysis of supplies.
- The team providing care during labour and delivery includes Obstetricians, Midwives, Anaesthesiologists, and Neonatologists. All of them should be trained about all the preventive measures.
- Whenever possible, dedicated health care providers should be designated to care for known or suspected COVID-19 patients. Moreover, based on the obstetrics conditions, women should be assisted by the minimum required number of HCP limiting traffic around the room.
- Maternal vital signs monitoring should continue. Oxygen saturation should be checked every hour and should be > 95%. (19)

- Foetal heart monitoring should be continuous since the high rate of foetal compromise reported in pregnant women with COVID-19. (19)
- If the caesarean section is needed, it must be performed in a specifically designated operation room and by the same team providing patient care during labour.

It is unknown if the delivery can provide some benefit to a critically ill mother with COVID-19. Therefore, the decisions regarding timing of delivery should consider the gestational age, balancing the risks and benefits (29). About COVID-19, despite the scarcity of available data, it seems that caesarean section should be performed only based on obstetric indications. However, for the most severe cases of SARS in pregnancy, caesarean delivery and general endotracheal anaesthesia were elected in order to avoid emergency airway issues and to minimize exposure risk for HCP.(30) The same could be considered also for COVID-19 critically ill patients.

Moreover, both general and locoregional anaesthesia could be used during caesarean section in pregnant women with COVID-19, as well as neuraxial analgesia is allowed for intrapartum pain control.(30) In particular epidural analgesia should be offered and recommended early in labour in order to reduce the need of general anaesthesia if urgent/emergent delivery was needed, this allows to reduce the risk of virus spreading via aerosol associated with Entonox breathing system use.(19)

4. Vertical transmission of SARS-CoV-2.

Whether a pregnant woman with COVID-19 can transmit SARS-CoV-2 to her foetus or neonate by vertical transmission is still unknown. A report of 18 pregnant women with confirmed COVID-19 or under investigation shows that there is no evidence of a positive laboratory test that proves vertical transmission to the newborns.(27,28) Similarly, experience from SARS and MERS in pregnancy shows no confirmed intrauterine coronavirus transmission from mother to fetuses (35). Moreover, a report of three cases has recently been published giving data on clinical characteristics and placental pathology of SARS-CoV-2 infection in pregnancy. From Pathological studies, no morphological

changes were found in the placentas and all samples were negative for the nucleic acid of SARS-CoV-2. Given the importance of this information in understanding the modality of virus transmission, it is important to send for pathological investigation any product of conception (36). Additionally, data from infections in newborns can give an insight in the mode of transmission. Three cases of infection in newborns have been reported. The first one has been diagnosed with COVID-19 when he was 17-day-old, after many close contacts with his mother and grandmother, both confirmed with COVID-19. The second one was diagnosed 36 hours after he was born; however, the possibility of close contact history cannot be ruled out and the way and timing of infection are still unclear. A third case was diagnosed 30 hours after birth suggesting the possibility of in utero transmission. However, insufficient information is available to rule out perinatal or postnatal modes of transmission (27). Based on these cases, it is more likely that the babies have been infected after birth from the environment, instead of having a vertical transmission (35). However recently a research letter published on JAMA by Dong, L. et al reported a new neonatal case where elevated IgM antibodies (that don't cross the placenta) to SARS-CoV-2 have been found in the newborn two hours after birth. The mother had been diagnosed with COVID-19 23 days before the delivery. This result could cast doubt of possible vertical transmission. Despite this RT-PCR for viral RNA on neonatal swab tested negative. At the moment there is no evidence that delayed cord clamping should be avoided after birth, except for other indications. Moreover the baby can be dried and cleaned as usual, while the cord is still intact (19). From SARS guidelines on neonatal management it was only indicated that the baby should be rapidly cleaned from maternal fluids (37).

5. Breastfeeding

What we know from pandemic H1N1 and from available Chinese literature on COVID-19 is that temporary separation of the infant from the mother has been adopted in order to reduce the risk of transmission to the baby. This can be considered in some cases, but no data are available to guide the length of separation. However, the

decision to adopt a routine precautionary separation has a relevant impact on bounding and feeding, and the risks and benefits must be balanced. Indeed, breast milk is the best source of nutrition for most infants and provides protection against many other illnesses. Moreover, the virus that causes COVID-19 has not been found in colostrum of women with COVID-19; conversely, antibodies anti-SARS-CoV were found in at least one case (27,33,38). On that basis, given the most likely mechanism of transmission and the available data, some authors suggest that breastfeeding benefits outweigh potential risks of transmission of the virus through milk.(19,29) Moreover, in the case of temporary separation of the infant from the mother, breast milk should be favoured if allowed by maternal clinical conditions.

The Royal College of Obstetricians & Gynaecologists has recently published advices for the postnatal management. Given the limited evidence, the mother and the healthy infant should be kept together in the immediate postpartum period, unless other reasons for separation are present (19). The mother should be informed on the benefits and risks of breastfeeding (linked to the close contact rather than to milk itself from what we know so far) and should also be instructed on the hygiene measures that must be adopted to reduce the risk of transmission. A mother with confirmed COVID-19 or who is a symptomatic person under investigation should take all possible precautions to prevent infant exposure, including washing her hands before touching the infant and wearing a face mask, if possible, during breastfeeding. In the case of breast milk with a manual or electric breast pump, the mother should wash her hands before touching any pump or bottle parts and follow recommendations for proper pump cleaning after each use (33,39).

In general, whether and how to start or continue breastfeeding should be determined by the mother in coordination with her family and HCP. Breastfeeding could be considered in women with confirmed or suspected COVID-19 with mild symptoms if they wish (33). The possible decision for separation, when appropriate, should be taken based on the benefits and risks related to the separation in consultation with infectious control experts and neonatologists (29). In any case, a baby born from a mother with

suspect or confirmed COVID-19 should be observed closely and should be tested for COVID-19 (19).

Regarding home care, it can be considered for mothers after delivery in the case the residential setting is suitable for outpatient management of COVID-19. Consultation with an Infectious Disease Specialist is suggested also to understand specific management of the neonate at home in order to reduce the risk of trans-mission.

6. Quarantine

Quarantine has been reported having a psychological impact both for patients and HCP (24). Alcohol abuse, dependence symptoms, and avoidance behaviours have been reported associated with the experience of quarantine as well as to the work in high-risk areas (24). Short and long term negative psychological effects, psychological distress, and disorders have been reported (24).

On that basis, the implementation of mitigation measures is of paramount importance for both HCPs and patients. Noteworthy, pregnant women have been reported as the category of patients caring more about the risk of getting infected or becoming a source of infection for others in the setting of epidemic spread of infections (40). On that basis, appropriate counselling about the impact of SARS-CoV-2 infection and COVID-19 in pregnancy is of paramount importance in this category of patients.

DISCUSSION

At the end of 2019, the novel SARS-CoV-2 was identified as the cause of some cases of pneumo-

nia. Today, the number of cases is growing worldwide due to a widespread diffusion of the virus and probably the reported numbers are likely underestimated. In this scenario, the application of public health interventions is mandatory to limit the spread of the infection. Since very little is known about effect of COVID-19 on pregnant women and infants, a pressing need has emerged to gather information specific to the maternity setting. Noteworthy, the management of an obstetric patient can be more challenging given the characteristics of this special population and limited reported data. For these reasons, after thorough consultation of the literature and public health authorities and scientific societies guidance documents, we outlined and reported here a procedure and recommendations for the management of the obstetric and gynaecologic patient approved by a multidisciplinary team. We obviously acknowledge that current knowledge on this issue is provisional, incomplete, and therefore subject to change as new evidence becomes available.

DISCLOSURE STATEMENT

The authors have no proprietary, financial, professional or other personal interest of any nature in any product, service or company. The authors alone are responsible for the content and writing of the paper. All the authors conform the International Committee of Medical Journal Editors (ICMJE) criteria for authorship, contributed to the intellectual content of the study and gave approval for the final version of the article.

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Anti-Müllerian hormone: clinical implications in Gynecological Endocrinology. An update review

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ABSTRACT

Anti-Müllerian hormone (AMH) is produced by the granulosa cells of the ovary with serum levels that grow until puberty, remain stable up to 30 years and then begin to decline until menopause. It is mainly produced by pre- and early antral follicles with an average diameter of 5-8 mm and it indirectly represents the ovarian reserve (OR). The purpose of this review is to identify what can currently be done with AMH, according to the most recent scientific evidence. AMH does not appear to be a marker for fertility as it does not reflect the quantity but not the quality of follicles. It is not able to predict the spontaneous onset of pregnancy, nor the pregnancy rate in cycles of assisted reproduction technology (ART) but is a good predictor of ovarian response to hyperstimulation and it is useful in planning a couple's fertility treatment even in the case of women undergoing chemotherapy, radiotherapy and ovarian surgery. It helps to identify women suffering from mild forms of polycystic ovary syndrome (PCOS) and diagnose and manage menopause and premature ovarian failure (POF). Finally, AMH levels may be used in case of granulosa cells tumors, both for diagnosis and follow up after surgery.

SOMMARIO

L'ormone anti-Mülleriano (AMH) viene secreto dalle cellule della granulosa dell'ovaio ed i suoi livelli sierici aumentano fino alla pubertà, rimangono stabili fino a 30 anni e successivamente si riducono fino alla menopause. Viene prodotto principalmente dai follicoli pre-antrali ed antrali con diametro medio di 5-8 mm e rappresenta indirettamente la riserva ovarica. Lo scopo di questa review è quello di far emergere l'utilità clinica dell'AMH in accordo con le più recenti evidenze scientifiche. Attualmente l'ormone non sembra un adeguato marker per la fertilità, poiché rappresenta solo la quantità e non la qualità dei follicoli. Non è in grado di predire l'insorgenza spontanea di gravidanza né il tasso di gravidanze nei cicli di riproduzione medicalmente assistita, ma è utile nel predire la risposta ovarica alla stimolazione e nel programmare i percorsi di assistenza alla coppia infertile, anche in caso di donne sottoposte a chemioterapia, radioterapia ed interventi chirurgici sull'ovaio. Inoltre, l'AMH è utile per diagnosticare forme lievi di sindrome dell'ovaio policistico (PCOS), individuare e gestire i casi di esaurimento ovarico prematuro e può aiutare nel predire l'insorgenza della menopause. Infine, l'ormone può essere utilizzato nella diagnosi e nel follow-up post-chirurgico dei tumori delle cellule della granulosa.

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Key words: Anti-Müllerian hormone (AMH); ovarian reserve, pregnancy; infertility; ovarian; dysfunctional diseases; assisted reproductive technology

INTRODUCTION

AMH, which has been known since the 1940s, is a dimeric glycoprotein member of the transforming growth factor- β (TGF- β) superfamily. It has a role in sexual differentiation: it is produced by Sertoli cells of male foetuses and induces regression of the Müllerian duct, allowing the formation of the male reproductive tract from the Wolf ducts (1). Instead, the absence of AMH allows the differentiation of the Müllerian duct into the oviduct, uterus and upper vagina. Subsequently, the hormone is produced by the granulosa cells of the ovary from around 36–38 weeks of gestation (2), with serum levels that grow until puberty, reaching the peak at 20–25 years, and then decline from 30 years up to menopause (3). By immunohistochemistry of human ovarian tissue, it emerged that AMH is mainly produced by primary, secondary, pre-antral and early antral follicles and 60% of the serum AMH comes from follicles with an average diameter of 5–8 mm (4). Instead, follicles greater than 10 mm produced a very small amount of the hormone. Moreover, AMH inhibits follicle response to follicle-stimulating hormone (FSH) and this suggests that it has a role in controlling follicles recruitment (5–7). Indeed, experiments with AMH knock-out mice showed an increase in primordial follicles recruitment and their premature depletion, and lower serum FSH levels, suggesting an increased sensitivity of follicles to this hormone (8). Furthermore, AMH is produced mainly by pre-antral and early antral follicles, which are proportionally related to the primordial follicle pool, so it indirectly represents the ovarian reserve (9,10). This can also be assessed by antral follicle count (AFC) with transvaginal ultrasound, which has a strong positive correlation with AMH levels (11,12). In fact, both have the ability to accurately estimate the pool of ovarian follicles (13). However, AFC is highly dependent on the operator and it has inter- and intracycle variability (14). Moreover, women may perceive it as more invasive than a blood sample. Instead, AMH can be dosed any day of the menstrual cycle, during pregnancy or during a period of amenorrhoea because it is FSH independent (14,15). Although, in women who use oral contraceptives it is still debated whether the values of AMH are modified by these or not (16). In literature, the role of AMH as a predictor of

ovarian reserve and response to ovarian hyperstimulation in women undergoing ART is well known (17). Despite this, it does not seem to have the capacity to assess the quality of oocytes nor to predict the likelihood of pregnancy occurrence. Other hypothesis on the use of AMH have been proposed in the literature, such as its role in the diagnosis of PCOS, in the prediction of premature ovarian failure and evaluation of ovarian reserve in women undergoing chemotherapy. But what can we actually do with AMH, according to current scientific evidence? The purpose of this review is to answer this question through a careful analysis of the most recent literature on the subject.

AMH FOR PREDICTING SPONTANEOUS PREGNANCY CHANCES IN WOMEN WITH AND WITHOUT INFERTILITY

As we have already said AMH reflects the quantity and not the quality of ovarian follicles, but the OR is represented by both these characteristics (18). That is why many authors have critically analysed its role in predicting the spontaneous onset of pregnancy. One of the most important issues is the absence of a cut-off value of AMH under which we can say with certainty that a woman cannot have a pregnancy. In fact, even if there is a negative association between AMH level and time to pregnancy in fertile women, there is also a wide variability in fecundity in women with similar hormone concentration (19). A recent prospective cohort study has analysed the time to pregnancy in 30–44 years old women without a history of infertility, who had been trying to conceive for 3 months or less, and found no correlation between biomarkers of diminished ovarian reserve, like low AMH and high FSH, and reduced pregnancy rate (20). However, in the study is stressed that they do not look at AMH values lower than 0.1 ng/ml, which reflect a more consistent drop in OR and may negatively affect fecundability. Another study, including 20–35 years old women, found no significant reduction in the pregnancy rate in women with AMH values < 1.4 ng/ml compared to those with higher values (21). Different recent studies reached the same conclusion (22,23) and according to a review by Dawailly and Laven (24) AMH is not a primary marker for

fertility and some authors claim that both AMH and AFC are just expression of woman's "ovulatory potential" (25). Even in women undergoing ovarian surgery for benign ovarian cysts it seems that AMH doesn't have a role in predicting the pregnancy rate. In fact, a study conducted in our department has analysed the pre- and post-operative AMH values of these women and evaluated the spontaneous onset of pregnancy of those who tried to conceive (26). We obtained a live birth of 37% and found no statistically significant difference in reproductive outcome between women with AMH serum levels lower and higher than 1.1 ng/ml. The same result was found also by another study, even with the same live birth rate (27). Moreover, the study conducted in our department found out that AMH levels decline 6 months after surgery in both women with endometrioma and in those with other benign ovarian cysts, but only in case of endometriotic cysts this decline was statistically significant. Anyway, there is a statistically significant recovery at 12 months in women with endometriotic cysts.

Many studies have analysed the general population but only a few observed the AMH role in case of unexplained infertility. One of these, conducted in our department, has included 83 women with unexplained infertility with normal or low ovarian reserve and observed the spontaneous onset of pregnancy and found that markers of OR are similar between women who get pregnant and those who don't (28). Moreover, in the aforementioned study were obtained 5 pregnancy in women with AMH lower than 0.4 ng/ml, a value diagnostic for abnormal ovarian reserve, one of the three diagnostic criteria for Poor Ovarian Response (POR), confirming that POR does not mean sterility and women in this condition could get pregnant (29,30).

An interesting observation is that 2 of these 5 pregnancies resulted in miscarriage and in fact another recent study hypothesized a role of AMH as a risk factor for miscarriage in spontaneous pregnancy (31). They included 460 pregnant women and after adjusting for age, BMI, race and history of recurrent pregnancy loss, they found out that the risk of miscarriage decreased as AMH increased and women with AMH ≤ 0.4 ng/ml have over twice the risk. Other studies confirm this hypothesis and observed that women with an unknown cause of

miscarriage have a significantly lower AMH than those with an identifiable cause of pregnancy loss (32,33), so AMH is not a primary marker for fertility but it could be a marker of lower reproductive potential (31). However, there are many other factors that can affect fertility, such as body mass index (BMI), smoking, age and other diseases which we should study more carefully (19).

AMH AS A MARKER FOR THE DIAGNOSIS OF PCOS

PCOS has a prevalence of 8-13% in reproductive age women (34-36) and it is one of the most common gynaecological endocrine disorders, characterized by a wide variety of symptoms.

The diagnosis of PCOS is based on the presence of two out of three Rotterdam Criteria, which are oligo-anovulation, polycystic ovaries (PCO) on ultrasound and clinical and/or biochemical signs of hyperandrogenism (HA) (37), but this criteria are not always easy to apply. For example, hyperandrogenism is difficult to define because of interobserver variability of Ferriman-Gallwey hirsutism scoring system (38) and the poor reliability of androgen assay (39). Moreover, 20-40% of women with PCOS have normal androgen levels, in fact some authors wonder if clinical and/or biochemical hyperandrogenism should always be present to diagnose PCOS, since the serum levels of AMH and the number of follicles are considered a substitute for the expression of hyperandrogenism (40).

The reliability of the ultrasound diagnosis of polycystic ovary is also controversial, because it depends strongly on the equipment and the operator, it is not easy to reproduce and it is more invasive than a blood sample. Furthermore, the improvement in ultrasound technology has led to the revision of the previous cut-off value of follicles number to diagnose PCO (41), which could further change in the future. Recently several studies have pointed out that AMH could play a crucial role in the diagnosis of PCOS and may help improve the diagnostic capacity of Rotterdam Criteria. In fact, this hormone is significantly higher in women with PCOS than in healthy women (42,43), reflecting the increased number of early antral follicles.

Furthermore the serum levels of AMH are

positively correlated with the severity of the disease (44).

It also appears that AMH may play a role in the pathogenesis of PCOS, passing through the placenta and influencing embryo development (45,46). Also, as we have already said, AMH is correlated with AFC and also with biochemical hyperandrogenism and according to some authors one can be used instead of the others (47-51). Two studies conducted in our department have analysed the role of AMH in the diagnosis of PCOS, concluding that AMH is more reproducible than AFC and that it can help to identify those women suffering from mild forms of PCOS, better than using only the Rotterdam Criteria (52,53). One of the two studies also identified a serum AMH value for the diagnosis of PCOS equal to 33 pmol/l (4.62 ng/ml), able to predict PCOS with a sensitivity of 95% and a specificity of 95% (52).

The other study conducted in our department also noted that the AMH can help in the diagnosis of the PCOS by reconciling the Rotterdam criteria with the other two often used criteria, those of National Institutes of Health (NIH) and the Androgen Excess and PCOS Society (AE-PCOS), reducing the difference in prevalence of diagnosed PCOS with these different criteria (53). A recent review has analysed several studies that have searched for an AMH cut off value for the diagnosis of PCOS, concluding however that there is too much heterogeneity in the accuracy of AMH in reflecting PCO and in helping the diagnosis of PCOS (54). It also depends too much on age and there is a need for specific cut offs for each age group (55).

Moreover, AMH seems to be more useful in PCOS diagnosis in adults than in adolescents because the hormone levels are higher at this young age, both in the case of girls with PCOS and those who do not have the syndrome (54). This also reflects the controversial use of ultrasound in adolescents for the evaluation of PCO, which is closely related to AMH. In fact the new international guidelines do not recommend ultrasound evaluation in the diagnosis of PCOS until 8 years post-menarche (33-35).

Therefore, AMH can be helpful in the diagnosis of PCOS together with the Rotterdam criteria, but there is a need to standardize AMH assays, improve their accuracy and to identify age specific AMH level cut-offs.

AMH AND ART

Currently AMH is reliably used to predict ovarian response in women undergoing ovarian stimulation for IUI and IVF (56). In fact AMH is a good predictor of ovarian response to ovarian hyperstimulation (10, 57). This led to the use of algorithms to find the right dose of stimulation based on the initial AMH value, reducing both the risk of ovarian hyperstimulation syndrome (OHSS) and POR (58-60). It has also been hypothesized that in women with PCOS AMH may be useful in deciding the initial dose of stimulation to start with, in fact some authors argue that if AMH is high then stimulation should be started with higher doses (61). On the contrary, it seems there is no correlation between AMH serum levels and gonadotropin sensitivity in patients with PCOS, and the only real role of AMH in these patients is to predict the risk of hyperstimulation syndrome, to which they are more exposed (24). Moreover, a recent Cochrane review show that current evidence does not provide a justification for adjusting the standard dose of FSH in the case of poor or normal responders, indeed this would only lead to using a higher total dose of FSH and increasing the costs of stimulation. Instead, a decreased dose in predicted high responders may reduce the risk of OHSS (62). However, like in the case of spontaneous onset of pregnancy, it seems that AMH has little ability to predict pregnancy chances in women undergoing ART (57).

A recent meta-analysis points out that, although AMH levels could be useful in planning a couple's fertility treatment, its predictive accuracy for pregnancies is poor (63). This is because even if AMH has a correlation with the number of follicles that could be fertilized there is no correlation with oocytes and embryos quality (64-66). However, in literature there is still debate on this topic and some studies claim that AMH has a predictive ability for the pregnancy rate (63,67-76), while others do not (77-81). A recent study evaluates the ability of AMH levels, stratified by age, to predict live birth rate in IUI and it concluded that it has a poor predictive value, although they have found a tendency for AMH to be lower in cases of miscarriage and lower pregnancy rates, suggesting that the reduction of the ovarian reserve could be a quantitative but also a qualitative problem (82).

In any case, regardless of the debate in literature, it is certain that the two main factors that influence the success of ART procedures are age and AMH levels (75,83-85) but no AMH value cut-off was identified under which pregnancy was excluded and many different studies have obtained pregnancy with ART in women with very low AMH concentration levels (80,86-88). In conclusion AMH can strongly predict poor ovarian response in ovarian stimulation (89) and it is a useful tool to schedule fertility treatments, but it has little power in predicting pregnancy rate.

MENOPAUSE AND POF PREDICTION WITH AMH

Menopause is defined as the point in time that follows 1 year after the complete cessation of menstruation (90,91), the average age at which it occurs is 51 years, with a range of 40-60 years (92). If cessation of menstruation occurs before age 40 it is called premature ovarian failure or POF (90) and affects 1‰ women of 15-29 years and 1% of women from age 30 up to 39 (93-95). Risk factors are multiple and include hereditary diseases, autoimmune diseases, smoking, alcohol, chemotherapy, ovarian surgery, viruses and others (90,92). POF is diagnosed by two dosage of serum FSH levels, 1 month apart from each other, that measure greater than a threshold range of 30 to 40 mIU/mL (90). The AMH is related to the ovarian reserve and its serum levels decrease with the decrease of this one, therefore it has been hypothesized that it can be used to predict the time of onset of menopause and POF (92,94,96-98). It would be really useful if those hypotheses were confirmed as this could influence women to have pregnancies before the possible cessation of menstrual cycles or apply fertility preservation techniques such as oocyte freezing. In fact, a recent study questioned women about the possibility of performing blood sampling to dose AMH to predict the onset of POF and menopause and women expressed a positive opinion, especially in the case of familiarity for POF (99). Unfortunately, nowadays even if the presence of constant low levels of AMH is a good marker in the diagnosis of POF (92,96,98), the recent developed model to predict the onset of POF and menopause has little accuracy (97,100). In addition, this model involves multiple serial doses over time and

requires reliable laboratories to perform the test (101). Moreover, the main issue consists in the absence of a widely accepted cut-off value in AMH serum level to diagnose a decline in ovarian reserve (24). In literature many authors suggest different thresholds, such as 1 ng/ml, but since there is evidence of pregnancies occurring even in women with undetectable levels of AMH, is clear that those thresholds do not predict the chances of spontaneous pregnancy (102,103). A recent study compared the risk of early menopause associated with AMH levels of 1.5, 1.0 and 0.5 ng/ml to an AMH levels of 2.0 ng/ml found out that the risks were respectively 2.6, 7.5 and 23 (104). This highlights that surely the AMH can help in understanding if a woman is going towards the cessation of ovulatory activity but it is not able yet to identify a certain value below which a woman can't get pregnant. Progress in this field could be helpful also in women with Turner syndrome, who are destined to develop POF, since AMH has been found to be higher in those affected women who achieve puberty and represents a marker of the presence of follicle in their biopsied ovarian tissue (105,106). Hence, further studies are crucial to assess if AMH levels dosed at a young age could be used to schedule fertility preservation or pregnancy attempts (10).

AMH ROLE IN MANAGEMENT OF WOMEN UNDERGOING CHEMOTHERAPY, SURGERY AND RADIOTHERAPY

Along with the increase in cancer survival rate after therapy, the need to improve the quality of life of people who survived has also increased. This topic is very important for women of reproductive age, because it is known that radiotherapy, chemotherapy and surgery damage the ovary (107). AMH could be a useful tool in many aspects of cancer treatment and its outcome. Regarding chemotherapy, the use of the hormone to define the decline of the ovarian reserve has been studied for the first time on childhood cancer survivors (108), find out that they have lower serum AMH levels compared with healthy women. A recent study analysed a group of childhood cancer survivors after 10 years, being in their mid-thirties, showing a decrease in AMH levels according to the gonadotoxic effect of the treatment to which they were exposed

(109). In fact the decrease in AMH level was used to establish the ovarian toxicity power of chemotherapeutic agents (110,111).

In women undergoing treatments for breast cancer AMH levels drastically decrease during chemotherapy, becoming undetectable after six cycles of therapy in most women (112), but it seems that there is a limited recovery after 3-6 months in some cases (113). The damage to the ovarian reserve depends on the type of chemotherapy agent and the age at which the therapy started. For example alkylating agents are associated with the highest risk of gonadotoxicity, amenorrhoea and lower recovery of AMH serum levels (114,115). The ovary is also very sensitive to radiotherapy and the damage depends on irradiation field, therapy dosage, fractionation schedule and whether the patient is pre or post menarche (116), but there are few data about changing in AMH level after radiotherapy. However the hormone could play a role in the management of women undergoing chemotherapy and radiotherapy, especially in deciding whether there is a need to apply fertilization preservation techniques (117,118). The pre-therapy levels of AMH are useful for predicting the loss of ovarian activity, especially when combined with age, providing useful information to plan the available fertility options with the patient. In fact higher levels of AMH before therapy combined with younger age brings a lower risk of chemotherapy-related amenorrhoea (112,115,119) and higher chance of restoration of normal ovarian function (120,121).

Moreover, the higher the pre-treatment AMH, the faster it rises again after therapy (122). Instead AMH recovery is lower in older aged women (123). Thus, in cases where the risk of iatrogenic POF development is high, ovarian tissue cryopreservation can be chosen. Regarding surgery for benign ovarian cysts or endometriomas, it has emerged that AMH undergoes a decline 3-6 months after surgery (26,124), but it seems to be statistically significant only in case of endometriomas and the hormone is unable to predict whether an operated woman may or may not have a pregnancy in the future. Furthermore, in the case of endometriotic cysts there was also a recovery of the AMH values at 12 months from the operation (26,125,126). Salpingectomy does not affect ovarian reserve, while unilateral salpingo-oophorectomy obviously

lead to a decline in AMH levels, there are few studies that analyse if women with history of unilateral salpingo-oophorectomy experienced an accelerated loss of oocytes and a premature loss of fertility (127). In any case, as we have already said, we cannot use AMH to predict the pregnancy rate and we cannot predict if a woman, even with very low AMH values, will get pregnant. For example, in case of orthotopic transplantation of ovarian tissue AMH levels are undetectable in most women, probably because of a poor vascularization of the transplanted tissue and a loss of follicles during the procedure of implantation, but pregnancies still have been reported anyway (128,129). Moreover, AMH levels may be used in women with granulosa cells tumors, both for diagnosis and for follow up after surgery, because this cells secrete the hormone (130).

Finally, there is a new hypothesis about the role of AMH in the therapy of epithelial ovarian tumors, given its ability to induce the regression of müller duct cells in the foetus (131).

CONCLUSION

In conclusion AMH reflects the ovarian reserve in terms of quantity but not quality of ovarian follicles. It is a useful tool to predict ovarian response to hyperstimulation in women undergoing ART and it can be used to decide the right dose of FSH to start with in order to avoid OHSS or POR. It has a crucial role in the diagnosis of mild cases of PCOS, when using only Rotterdam criteria is not conclusive. In women of late reproductive age, as in those with familiarity for POF, it can help to predict the onset of menopause, although it does not estimate the likelihood of pregnancy in these women. In the case of ovarian surgery, chemotherapy and radiotherapy, pre-treatment serum levels of AMH can help decide whether to consider techniques such as ovarian tissue cryopreservation, while post-therapy levels may indicate damage to the ovarian reserve. In tumors of granulosa cells that secrete AMH, it can be used in diagnosis and post-surgery follow-up. Moreover, new pathways are being studied to use AMH as a therapeutic agent in epithelial cell tumors. In any case, AMH levels are never able to predict a woman's chance of getting pregnant because, accordingly with the most recent literature, it is not marker of fertility

and many cases of women who achieved a pregnancy with very low AMH values were observed. However, further studies are needed

especially to identify a reliable assay to be used in all laboratories to make the AMH values, obtained in the different centres, comparable.

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Electrochemotherapy in vulvar cancer: a systematic review

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ABSTRACT

There are some problems about the optimal treatment modality of vulvar cancer (VC): unfeasible surgery in elderly women with several comorbidities and the absence of treatments applicable in recurrent disease. For these reasons, Electrochemotherapy (ECT) may have an important role in the management of these patients. The aim of this systematic review is to evaluate ECT in VC in terms of clinical response, adverse events and quality of life (QoL). We conducted a search on the electronic database PubMed/MEDLINE. All the studies in English language published from 2006 and August 2019 were considered eligible. The 4 studies included in the systematic review reported an overall objective response rate (complete and partial response) was 74.3%. No treatment-related serious adverse events were reported in any of the studies. An improvement in the QoL was reported. In conclusion, ECT is an easy, quick to perform, less invasive and repeatable procedure, which have shown a positive clinical response, a reduction in symptoms and an improvement in QoL. Since the survival for advanced and metastatic diseases has been, fortunately, increased, it is also important to focus our efforts on the QoL and on the local control of the disease.

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SOMMARIO

Alcune delle problematiche sul trattamento ottimale del carcinoma vulvare (CV) sono rappresentate dalle controindicazioni all'esecuzione di un intervento chirurgico nelle donne anziane con comorbidità e dall'assenza di ulteriori trattamenti somministrabili nella malattia recidivante. Per questi motivi, l'elettrochemioterapia (ECT) potrebbe avere un ruolo importante nella gestione di queste pazienti. Lo scopo di questa review sistematica è quello di valutare l'ECT in termini di risposta clinica, eventi avversi e qualità di vita (QoL). Tramite una ricerca su PubMed/MEDLINE, tutti gli studi in lingua inglese pubblicati dal 2006 all'agosto 2019 sono stati presi in considerazione. I 4 studi inclusi nella review sistematica hanno riportato una risposta clinica complessiva (completa e parziale) del 74.3%. Sono stati riportati, inoltre, l'assenza di gravi eventi avversi e un miglioramento della QoL. In conclusione, l'ECT è una procedura semplice, veloce, poco invasiva e ripetibile, che ha mostrato una risposta clinica positiva, una riduzione della sintomatologia ed un miglioramento della QoL. In un contesto in cui, fortunatamente, la sopravvivenza per le malattie avanzate e/o metastatiche sta aumentando, è importante concentrare i nostri sforzi anche sulla QoL e sul controllo locale di malattia.

Key words: *electrochemotherapy; vulvar cancer; palliative therapies*

INTRODUCTION

Electrochemotherapy (ECT) combines electroporation and chemotherapeutic drugs in order to destroy or reduce in size different kinds of lesions. The electroporation improves drugs delivery by increasing the cell membrane permeability and makes chemotherapy more effective (1). There are three mechanisms of action of ECT (2): first, the cytotoxic effect of chemotherapy; second, an anti-vascular effect with a vasoconstriction phase followed by vascular disruption (3,4); third, an immune stimulation (5,6). The applications of ECT have been extended in recent years. ECT has been used in the treatment of metastatic melanoma in conjunction with standard chemotherapy in order to obtain a better local control of the disease (7,8). Two recent multicentric studies reported 71% of positive response to the ECT treatment in breast cancer patients (9, 10). In head and neck cancers, ECT may be a valid less invasive option to preserve physiological functions (11). Furthermore, ECT showed positive results in non-melanoma skin cancers and soft tissues sarcomas and even in non-cancer skin lesions (2). More recently, promising results have been reported in vulvar cancer treatment. Vulvar cancer (VC) accounts for 5% of all gynaecologic malignancies (12) and there are some problems about the optimal treatment modality. Indeed, radical surgery often requires the need for plastic reconstruction, by different techniques (13, 14), and frequently involves a high post-operative burden (15). On the one hand, since vulvar cancer mainly affects elderly women, comorbidities often make surgery unfeasible, whereas on the other hand, even when surgery, chemotherapy and/or radiotherapy are applicable, in the different settings up to the palliative care (16, 17, 18), recurrences are not rare and to provide additional care remains a real challenge. For these reasons, ECT may have an important role in the management of these patients. The aim of this systematic review is to evaluate ECT in VC in terms of clinical response, adverse events and quality of life (QoL).

MATERIALS AND METHODS

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement (PRIS-

MA) was used as a guide for this systematic review (19).

The Population, Intervention, Comparator and Outcomes (PICO) framework was used to formulate a search question in the following way: P: vulvar cancer; I: electrochemotherapy; O: clinical response. The "C" was not included because of the absence of a comparator. Indeed, ECT is emerging in more recent years and it is used as a palliative treatment in the majority of the cases. For this reason, comparing ECT with other less effective or none treatment would be unethical. Although the first objective of this systematic review was the evaluation of the clinical response, we added two secondary endpoints of our research: the evaluation of the adverse events and the QoL. We conducted a search on the electronic database PubMed/MEDLINE, by using the following terms: "electrochemotherapy" OR "ECT" AND "vulvar cancer". All the studies in English language published from the publication of the Standard Operating Procedures in 2006 (20) and August 2019 were considered eligible. The inclusion criteria were: diagnosis of VC, studies in which at least one of the three endpoints (clinical response, adverse events and QoL) was evaluated, studies in which the ECT was used as adjuvant or exclusive treatment. Abstracts, communications, comments, reviews and non-English language studies were excluded. Since this study is a systematic review of the literature based on previous published articles, no ethical approval or patient consent are required.

RESULTS

The search yielded a total of 8 studies after duplicates were removed (**figure 1**). We excluded 4 studies because of the following reasons: published before 2006, reviews or ECT was not used as exclusive or adjuvant treatment. Thus, 4 studies were included in the systematic review. All the studies included were prospective and the total number of patients was 105. Patients' and studies characteristics are shown in **table I**. The median age ranged from 68 to 85 years. Although the most common histology of VC was squamous cell carcinoma, there were two studies that included Paget diseases and one vulvar melanoma. Up to 40% of the patients in each

Table I. Patients' and studies characteristics.

Author	Type of study	Patients	Age	FIGO stage				Histology	Previous treatments	Multiple lesions
				I	II	III	IV			
Perrone et al. 2013 [23]	prospective	9	84 ± 3.9	2 (22%)	3 (33%)	4 (45%)	0	SCC	8 (88.9%)	2 (25%)
Perrone et al. 2015 [21]	prospective	25	85 (66-96)	13 (52%)	8 (32%)	3 (12%)	1 (4%)	SCC	21 (84%)	8 (32%)
Perrone et al. 2016 [24]	prospective	10	68 (59-84)	4 (40%)	4 (40%)	1 (10%)	0	SCC 1 PD	8 (80%)	4 (40%)
Perrone et al. 2019 [22]	prospective	61	79 (39-85)	24 (39%)	3 (4.9%)	7 (11.5%)	2 (3.3%)	SCC 3 PD 1 Melanoma	45 (73.8%)	24 (39.3%)

Data are shown as median (range) or mean ± SD and n (%). SCC: squamous cell carcinoma. PD: Paget Disease

study showed multiple lesions at the first pre-treatment examination. The primary tumour FIGO stage was I in the majority of the patients (41%), whereas only 3 patients were in FIGO stage IV. The majority of the patients (78%) underwent previous treatments before ECT, which were surgery in most of the cases with or without adjuvant therapies such as chemotherapy and/or radiotherapy. Data about ECT administration and clinical response are shown in **table II**. All the studies used intravenous Bleomycin as chemotherapeutic drug. In most of the patients general anaesthesia was used and the duration of the procedure was 20-28 min. The median hospital stays after ECT ranged from 1 to 3 days. For the response evaluation, two studies used the World Health Organization (WHO) criteria, whereas the other two studies used the Response Evaluation Criteria in Solid Tumours (RECIST) and the time of the response evaluation was in most of the studies 1 month. As regards the response rate, the 4 studies reported the following results: 49 (46.7%) complete response (CR), 29 (27.6%) partial response (PR), 12 (11.4%) stable disease (SD) and 8 (7.6%) progressive disease (PD). The overall objective response

rate (CR and PR) was 74.3%. No differences in the clinical response emerged between previously treated and non-treated patients (21) and, in the same way, no variations in the response rate emerged according to the number or diameter of the lesions (21,22).

Furthermore, Perrone et al. showed that age, BMI, FIGO stage, site and histology did not influence the response to the treatment (22).

No treatment-related serious adverse events were reported in any of the studies. Some studies reported minor local side effects such as minimal blood loss, oedema, erythema, hyperpigmentation, skin ulceration and mild pain (**table II**) (21,22,23).

Only three studies evaluated the QoL and all of them reported a significant QoL improvement after treatment (23,24).

In particular, they showed a reduction in bleeding, odour, urinary discomfort and pain (23,24). Perrone et al. reported a symptomatic response rate of 78% and they did not find differences in symptom-free survival according to previous treatments or tumor diameter (21).

In the end, with a follow-up of 6-12 months the overall survival exceeded 50% (**table II**).

Table II. ECT, clinical response and adverse events

Author	CT	Administration	Anesthesia	Procedure time (min)	H stay (days)	Response				Response eval. criteria	Eval. time (mo)	Adverse events	Qol	Follow up (mo)	Survival
						CR	PR	NR/SD	PD						
Perrone et al. 2013 (23)	BLM	IV	general	20 +- 4 min	1	5 (62.5%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	WHO	1	-Minimal blood loss -oedema	yes	9.1 ± 3.5	50%
Perrone et al. 2015 (21)	BLM	IV	Local + sedation	28 min	1	13 (52%)	7 (28%)	3 (12%)	2 (8%)	RECIST	1	-Minimal blood loss -oedema	yes	7 (1-27)	-
Pellegrino et al. 2016 (24)	BLM	IV	-	20 (10-20)	-	2 (20%)	4 (40%)	2 (20%)	2 (20%)	WHO	1	none	yes	12	60%
Perrone et al. 2019 (22)	BLM	IV	48 (79%) general, 13 (21%) local		3 (0-8)	29 (52.7%)	17 (30.9%)	6 (10.9%)	3 (5.5%)	RECIST	2	-Erythema 20% -hyperpigmentation 6% -skin ulceration 1% -mild pain 24%	no	6	72%

Data are shown as median (range) or mean ± SD and n (%). ECT: electrochemotherapy. CT: chemotherapy. BLM: bleomycin. CR: complete response. PR: partial response. NR: no response. SD: stable disease. PD: progressive disease. Iv: intravenous.

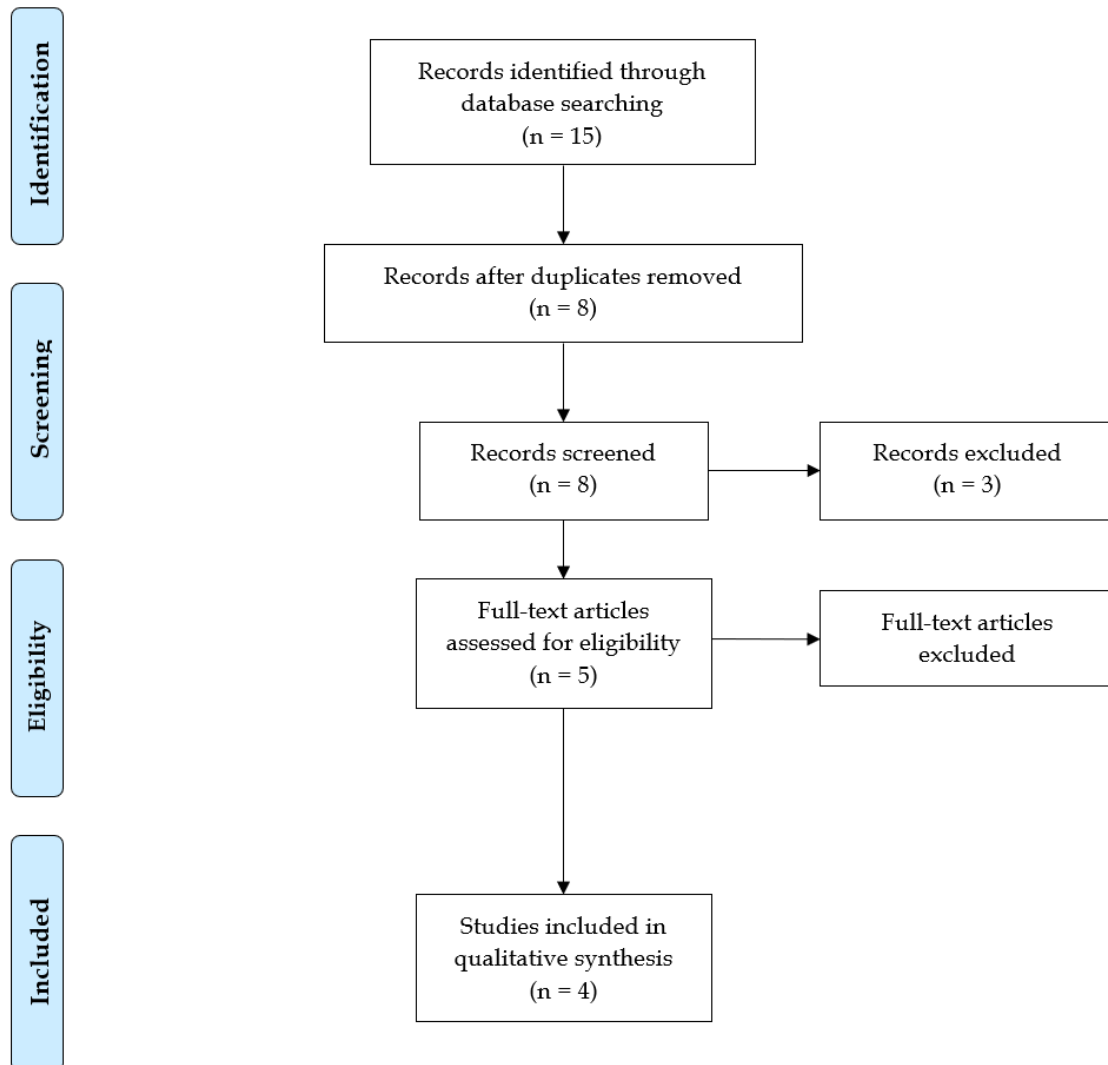


Figure 1. Flowchart for study selection and inclusion.

DISCUSSION

Modern oncology is increasingly characterized by minimally invasive therapeutic proposals (Interventional Oncology) (25,26). Especially the elderly (27,28) but also patients with comorbidity (29) can benefit from these types of procedures. ECT is part of this and it has demonstrated to be a valid option in the management of VC, showing a positive clinical response and an improvement of the QoL without serious adverse events. The studies we considered in our systematic re-

view were quite homogeneous. This is related to two reasons: on the one hand, the Standard Operating Procedures publication in 2006 and their update in 2018 (30) created a standardization of the procedure; on the other hand, the majority of the studies were conducted by the same institutions. Since the possibility of a duplication of the patients could not be avoided, it was not possible to add a meta-analysis, which is one of the limits of our research. Furthermore, the num-

ber of studies on ECT in VC is very low, because of the recent application of ECT in these kind of cancers. ECT in VC showed a CR rate of 46.7% and, although the response evaluation criteria were a bit different between the studies, the CR definition is the same. In a recent meta-analysis for the evaluation of ECT in different kind of tumors (VC not included), Morley et al. reported similar results, despite a higher objective response rate (82.2%) (31). Although some studies have reported a better response rate for tumors smaller than 3cm (31) and even the Standard Operating Procedures have suggested the possibility of different chemotherapy administration according to the tumour diameter (30), none of the studies in our systematic review found differences in the response rate (21,22). Furthermore, ECT response seemed not to be affected by any of the parameters considered in the studies (21,22). An interesting result was the absence of influence in the response rate when a previous treatment was administered (21), whereas some studies have found that treatment-naïve tumours responded better, probably because of a worse effect of ECT after radiotherapy, due to the reduction of the blood flow because of the fibrosis (31). As regards the survival outcomes, it is difficult to get conclusions because of the lack of homogeneity among the patients. Indeed, different previous treatments or the use of co-interventions may affect the survival outcomes. However, considering that ECT was more often used as a palliative treatment or when no other treatments were available, the survival outcomes showed by our research may be promising. The most common adverse events reported by our research were local: minimal blood loss, oedema, erythema, hyperpigmentation, skin ulceration and mild pain. Morley et al. showed minimal serious adverse events, whereas the most common were mild, post-procedural nausea and dizziness (31). Although ECT is associated with a 6% incidence of G3 toxicity, this rate is similar to other skin-directed therapies (2). All the studies we considered in our systematic review agreed with the fact that ECT is associated with an improvement in QoL. In a cohort of 52

patients, Campana et al. reported positive results of ECT in melanomas on local disease-related complaints such as wound healing, bleeding or pain and on activity of daily life (32). In head and neck cancer, which is a site where maintaining physiological functions is very important, ECT showed an improvement in physical functioning, role functioning and fatigue and pain reduction (33). Further applications of ECT include extra cycles of ECT and the use of this procedure as neoadjuvant treatment. First, few data exist on additional cycles of ECT (31). In VC, in two studies there were more than one ECT administration (21,24). Perrone et al. performed a second ECT cycle in a limited number of patients (20%) and they reported: CR 40%, SD 20% and PD 40% (21). Pellegrino et al. performed even more than two cycles of ECT and no complications occurred (24). Indeed, ECT have demonstrated to be a repeatable treatment, without increasing the adverse events rate and with similar results in clinical response. Second, acceptable aesthetic and functional results are often difficult to obtain in VC and, especially, in recurrent disease and extensive reconstructions are required (34,35). For this reason, ECT has been recently proposed as neo-adjuvant treatment, in order to reduce the tumor size and make the surgery less invasive and disabling. However, only one study evaluated this new application in a small group of patients with a positive objective response rate (77.8%) (36) and further data are needed. The few data we have on extra cycles of ECT seem to suggest that it maintains its safety and efficacy. For this reason, it can be able to obtain and/or maintain the tumour clinical response through repeated cycles of ECT (31). Furthermore, since the radiotherapy and surgery are often not repeatable and the chemotherapy role is debated in VC, according to the few but promising results of ECT as neo-adjuvant treatment, ECT may have also a theoretical role as an integrated procedure to standard surgical treatment techniques, although further specific studies are needed. Large-database using multi-centric system could help to develop predictive models (37) and decision support

tools that could be implemented in the clinical practice for improving the multidisciplinary discussing and for identifying the patients that may benefit by electrochemotherapy (38-40). In conclusion, ECT has demonstrated advantages in terms of reduction of hospital stay and costs. Furthermore, it is an easy, quick to perform, less invasive and repeatable procedure, which has

shown a positive clinical response, a reduction in symptoms and an improvement in QoL. However, few studies are present on VC and further data are needed.

Since the survival for advanced and metastatic diseases has been, fortunately, increased, it is also important to focus our efforts on the QoL and on the local control of the disease.

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Recurrence of aggressive angiomyxoma of the vulva: a case report

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ABSTRACT

Aggressive angiomyxoma (AA) of the vulva is a rare mesenchymal neoplasm that typically affects women of childbearing age, with a high rate of local recurrence. We present the case of a 38-year-old woman with local recurrence of aggressive angiomyxoma of the vulva 5 years after surgical excision of the first lesion. Aggressive angiomyxoma is diagnosed by histopathological and immunohistochemical features. The therapy is surgical, followed by a long follow-up or hormonal therapy to prevent recurrence.

SOMMARIO

L'angiomixoma aggressivo (AA) della vulva è una rara neoplasia mesenchimale che colpisce tipicamente le donne in età fertile, con un elevato tasso di recidiva locale. Presentiamo il caso di una donna di 38 anni con recidiva locale di angiomixoma aggressivo della vulva dopo 5 anni dall'escissione chirurgica della prima lesione. L'angiomixoma aggressivo si diagnostica mediante le caratteristiche istopatologiche e immunoistochimiche. La terapia è chirurgica, seguita da un lungo follow-up o terapia ormonale per prevenire la recidiva.

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Key words: *Angiomyxoma; vulvar neoplasms; mesenchymal tumor; case report; local recurrence*

INTRODUCTION

Aggressive angiomyxoma (AA) is a rare onset mesenchymal neoplasm that affects the pelvic and perineal region in reproductive age. It most frequently affects women in the fourth decade of life. It is a benign neoplasm first described in 1983 by Steeper and Rosai (1). It is characterized by a slow increase in volume, a high rate of local recurrence and a low tendency to metastasize (2).

CASE PRESENTATION

A 38-year-old woman, G1 P1 (one cesarean section), comes to our observation following the onset of vulvar neoforation on the large left lip of about 5 cm in diameter. The lesion appears of a soft consistency, movable on the superficial and deep planes and covered by apparently healthy

skin. The patient reports, in her anamnesis, about 5 years ago, excision of cysts of the left Bartolini's gland with histological outcome of "aggressive deep angiomyxoma". Performs MRI of the pelvic excavation which highlights "at the level of the large left lip, presence of an elongated aspect extending in an antero-posterior direction for about 6 cm, with maximum thickness at anterior level of 14 mm with significant contrast enhancement after administration of contrast agent. The patient, with suspected recurrence of aggressive angiomyxoma of the vulva, is therefore subjected to surgical exeresis of the vulvar neorfomation (**figures 1-3**). The histological examination confirms the diagnosis of aggressive deep angiomyxoma, with immuno-histochemistry of desmin positive neoplastic cells, smooth muscle actin (SMA), estrogen receptors, progesterone, CD34. The margins of resection are undamaged. It is therefore included in a six-year follow-up program for 5 years.

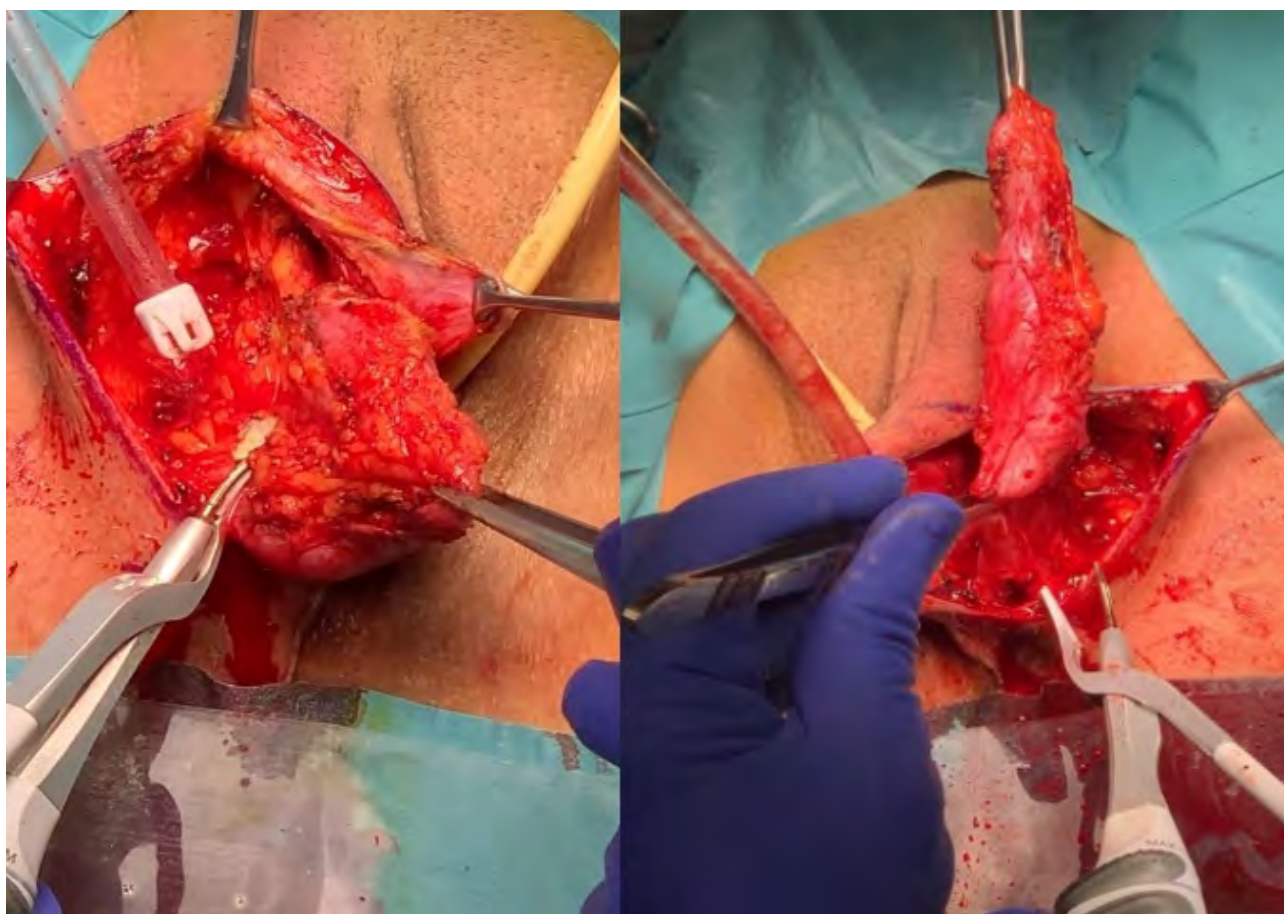


Figure 1-2. Surgical excision of the vulvar AA.



Figure 3. AA after surgical excision.

DISCUSSION

Aggressive angiomyxoma is a benign mesenchymal tumor that affects almost exclusively the genital, perineal and especially the vulva of women of reproductive age. It is characterized by slow growth but a high rate of recurrence even after several years after the first diagnosis (3). AA is mistakenly diagnosed in 80% of cases. Clinically it is, in fact, initially recognized as a cyst of the Bartolini's gland, lipoma, cyst of the small or big vulvar lip, Gartner duct cyst, etc. It enters in differential diagnosis also with neoforations such as superficial angiomyxoma, angiomfibroblastoma, cellular angiofibroma, leiomyofibroma and any polypoid neoformation of the perineum. In fact, it appears as a soft neoformation, such as to make the real evaluation of its volume difficult because of its consistency and its tendency to deeply invade the pelvic tissues, covered by healthy skin. It can be symptomatic and present with vulvodynia, dysuria, sense of weight or occasionally found during a gynecological check-up (4). Therefore the clinical diagnosis is very complex due to the rarity of this formation and the symptomatology common to other lesions of the genital sphere which

sometimes appears shaded or completely absent. For this reason it is possible to use different diagnostic methods for images that include ultrasound, Computed Tomography and Magnetic Resonance. Among these, Magnetic Resonance (MRI) plays a greater role due to the greater amount of information it provides (5). Indeed, MRI shows the isointense lesion in T1-weighted and hyperintense images in T2-weighted images due to the high-water content and the presence of free AA-typical mixoid matrix. It also allows a precise evaluation of the overall volume of the new formation and its limits with respect to the surrounding structures (6). The diagnosis is given by a set of histopathological features such as: stellate and spindle cells with poorly defined cytoplasmic margins and separated by abundant myxoid stroma and fibrillar collagen. The AA is also characterized by a well-represented vascular component with many thin vessels. There is no evidence of atypical mitotic activity or cellular atypia (7). Immunocytochemistry of the lesion is characterized by receptors positive for estrogens, progesterone, vimentin, desmin, SMA, CD34 and CD44 and always negative for S-100, carcinoembryonic antigen (CEA) and keratin. There is no consensus regarding the pathogenesis of this lesion. Recent cytogenetic studies have shown the presence of genetic alterations affecting chromosome 12, in the 12q13-15 region. In this region there is the HMHI-C gene (high mobility group protein isoform C) that encodes proteins involved in the regulation of gene transcription involved in the pathogenesis of AA. The possibility of using mutated anti-HMHI-C antibodies could be used to identify microscopic residue of neoplasia (8).

CONCLUSIONS

Therapy in the first instance is therefore excisional surgery, followed by follow-up or hormonal therapy. The hormonal treatment based on tamoxifen, raloxifene or Gn-RH analogues finds space in case of partially excised bulky lesions or in the treatment of relapses. Chemotherapy and radiotherapy are not useful in the treatment of these lesions due to the poor mitotic activity of the AA (9). Finally, it is im

portant to keep in mind that AA is characterized by a high rate of recurrence that can occur even after many years, so it is important to set a follow-up period of not less than 5 years.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Management of vertigo in pregnancy

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ABSTRACT

Background. Pregnancy is a specific female physiological period characterized by significant changes, included otological and neurotological manifestations. Vestibular disorders like vertigo and dizziness, are common complaints from pregnant women to primary care.

Objectives. The aim of this paper is to describe clinical pictures, evaluation methods and therapeutic options of acute vertigo in pregnancy with the related pathogenetic hypotheses.

Method. We describe 11 cases of vertigo in pregnancy. All patients underwent audio-vestibular evaluation, consisting of pure-tone audiometry, impedance and clinical testing of the vestibular function, the "bed side examination".

Results. Audiological evaluation showed normal pure-tone audiometry and impedance in 10 patients. Only in one case a sudden right total deafness was highlighted with vestibular areflexia, showing a secondary positional vertigo. Seven patients presented a benign paroxysmal positional vertigo (BPPV) effectively treated by liberatory maneuvers. Three patients had a diagnosis of vestibular neuritis and they were treated with corticosteroids therapy with a complete resolution of dizziness and vertigo in one month. **Conclusions.** Our results point out the importance of multidisciplinary between otolaryngologist, neurologist and gynecologist. From a pathogenic point of view, the vascular etiology, strictly related to the gravidic hormonal variations, is often hypothesized.

SOMMARIO

Razionale. La gravidanza costituisce un periodo di vita per la donna, caratterizzato da significativi cambiamenti di tipo fisiologico e adattativo, incluse possibili manifestazioni otoneurologiche. Disturbi vestibolari quali vertigini e instabilità, costituiscono una sintomatologia frequente nelle donne in gravidanza.

Obiettivo. Scopo dell'articolo consiste nel descrivere specifici quadri clinici, metodi diagnostici e opzioni terapeutiche di vertigine acuta in gravidanza con relative ipotesi patogenetiche.

Materiali e metodi. Descriviamo 11 casi di vertigine in gravidanza. Tutte le pazienti sono state sottoposte ad esame cocleovestibolare, ossia ad esame audiometrico tonale, esame impedenzometrico e esame vestibolare, clinico, la cosiddetta "bed side examination".

Risultati. All'esame audiometrico, 10 pazienti risultavano normoacusiche. Solo in un caso è stata riscontrata un'anacusia destra improvvisa associata ad areflessia vestibolare e successiva vertigine posizionale. Sette pazienti hanno presentato una vertigine parossistica posizionale benigna (BPPV) trattata con le manovre liberatorie. A tre pazienti è stata diagnosticata una neurite vestibolare, trattata con terapia corticosteroidica e conseguente completa risoluzione della sintomatologia in un mese.

Conclusioni. Alla luce dei risultati ottenuti, si dimostra fondamentale la multidisciplinarietà tra otorinolaringoiatra, neurologo e ginecologo. Da un punto di vista patogenetico, l'etiologia vascolare, strettamente correlata alle variazioni ormonali durante la gravidanza, è spesso ipotizzata.

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Key words: vertigo; pregnancy; vestibular test; benign paroxysmal positional vertigo; vestibular neuritis; otoneurology

INTRODUCTION

Pregnancy is a specific female physiological period, when symptoms and treatment of diseases should be carefully reasoned to avoid possible consequences to the child.

During this period significant physiological and adaptive changes occur in almost every organ and system such as cardiovascular, respiratory, haematological, renal, gastrointestinal, and endocrine for the development of the fetus and to prepare mother and child for the birth (1).

In particular, during the gestational period, various endocrinologic, metabolic, and physiologic changes might exacerbate otolaryngological manifestations such as patulous eustachian tube, nasal congestion, epistaxis, gingivitis, reflux and esophagitis (2). Moreover it is not infrequent that conditions affecting the ear and the hearing function could appear. Furthermore, concomitant pathologies that are not related to the pregnancy may arise.

During gestation otologic and neurotologic manifestations, which although transient, have important repercussions on the quality of life of women and affect the life routine involving family, social and professional environment thus causing a deterioration of physical and psychological well-being with the onset of frustration, depression and loss of self-confidence and concentration at work (3).

Vestibular symptoms include vertigo, unbalance, gait deviations, gait instability, feeling of floating, rotation and falls. However, during gestation, these same symptoms could be secondary to non-vestibular causes but they could be consequence of hormonal, anatomical and physiological factors affecting the musculoskeletal system occurring in pregnancy (4). The adaptation mechanisms occurring in the soft tissues, joints, and posture during pregnancy, cause an important discomfort with consequent pain, alteration of postural balance and increased risk of falling (5).

In literature there are only few articles about the care of ear problems in pregnancy and they consider just the management of hearing loss and tinnitus. Much less attention is given to the diagnostic and therapeutic procedure of the dizziness, despite they can be considered two important issues in the management of these patients.

Although there are few studies about dizziness and vertigo in pregnancy, they represent the most common complaints from pregnant women to primary care (6).

Probably this contrast is a consequence of the dizziness management in primary care that patients are rarely subjected to an otolaryngology examination because the dizziness is generally considered secondary to non-vestibular pathologies, so it's difficult for the otolaryngologists to define and study an adequate number of patients. Therefore otolaryngology specialists have little experience about management and treatment of vertigo in pregnant women and moreover, they are completely unaware of safety guide lines about the drug administration in pregnant. So patients are usually visited just by the gynecologist.

In this paper we describe different cases of vertigo in pregnancy and, in particular, the diagnostic approach we used to define the pathogenesis of dizziness and the possible autonomous and psychosomatic consequences.

MATERIALS AND METHODS

We describe a retrospective study of 11 cases of vertigo in pregnancy. Patients enrolled (31-42 years old, mean age 33.8, at gestational age 6-36 weeks, mean week 22.9) (**Table I**) came from the Obstetric Emergency Room to the ENT (Ear, Nose and Throat) Ambulatory Care Center of the Hospital.

All patients underwent audiological and vestibular evaluation, after otoscopy and complete medical history concerning symptoms and any comorbidities. History focused on related pathologies (hypotension or hypertension, cardiovascular pathologies, hypoglycemia, neuropathies, ophthalmological diseases), characteristics of dizziness, symptoms modifications by changes in head position or other triggers, oscillopsia (illusory movement of the environment), diplopia, neurological or otological symptoms (hearing loss, tinnitus, aural fullness, otalgia, otorrhea), onset and duration of symptoms, psychiatric contribution to the patient's symptoms and, moreover, information related to the gestational period. Audio-vestibular investigation consisted of pure-tone audiometry, impedance and clinical testing of the vestibular function.

Pure-tone audiometry (PTA) was obtained by averaging the air conduction thresholds at 0.125, 0.25, 0.5, 1, 2, 4 and 8 kHz and the bone conduction thresholds at 0.25, 0.5, 1, 2 and 4 kHz. A PTA between 20 and 40 dB (decibel) defined a mild hearing loss (HL), between 40 and 70 dB HL a moderate HL, between 70 and 90 dB HL a severe HL, and greater than 90 dB a profound HL. Audiological evaluation was completed by the execution of tympanometry and evaluation of acoustic-stapedius reflexes. The vestibular evaluation consisted of the bed side examination and the use of instrumental diagnostic when necessary.

At the bed side examination static vestibular balance was evaluated by identifying spontaneous nystagmus, vestibulo-spinal alterations (Romberg test, Fukuda test, star shaped march test, index finger test), otolithic signs (OTR- ocular tilt reaction-, skew deviation, ocular torsion -counter rolling- and head tilt). Dynamic vestibular function evaluation consisted of head impulse or Halmagyi test (HIT), Head shaking test (HST), positional tests (Semont, Dix Hallpike, Pagnini maneuvers), Vibratory test and Fistula test, Ocular motor testing (saccadic and smooth pursuit).

All procedures performed were in accordance with the ethical standards of our institutional ethical committee and all patients signed an informed consent.

Audiological evaluation showed normal pure-tone audiometry and impedance (tympanometry and evaluation of acoustic-stapedius reflexes) bilaterally in 10 patients (**Table I**). Only in one case the pregnant presented a sudden right total deafness with normal bilateral tympanograms and absence of right acoustic-stapedius reflexes.

The otoneurological examination showed the presence of several clinical signs of vestibular dysfunction (**Table I**). All patients underwent clinical neurological examination which resulted negative.

Analysis of the comorbidities demonstrated in 2 patients a condition of gestosis; one patient presented cardiac conduction disorder (first-degree atrioventricular block) and another patient was affected by type-2 diabetes mellitus and hypertension.

Another woman had a history of previous tran-

sient ischemic attack (TIA) already in ASA therapy, migraine with aura, heart disease (aneurysm of the interatrial septum with mitral valve prolapse and mild tricuspid dysplasia). There was also a case of patient with migraine and another one with migraine and celiac disease that referred the onset of vertigo after progesterone intake for amniocentesis. In another case the pregnant woman apparently had not risk factors for cocleo-vestibular syndrome, however, general examination and haematological tests demonstrated a condition of thrombophilia with increased D-dimer. This patient was treated with corticosteroids and anticoagulant therapy improving spontaneous vertigo in 4-5 days but without recovery of the auditory function. Positional secondary vertigo was efficacy treated by rehabilitative manoeuvres.

All patients with spontaneous nystagmus underwent neuro-radiological evaluation with contrast-enhanced magnetic resonance imaging (MRI) that resulted negative for pathological involvement (stroke, cerebral hemorrhage, brain tumors or cerebellopontine angle lesions).

RESULTS

Seven patients referred positional vertigo associated to neuro-vegetative symptoms (emesis) and presented positional nystagmus related to a benign paroxysmal positional vertigo (BPPV). In particular, in 4 patients posterior canal was involved (2 on the right and 2 on the left side) with a paroxysmal geotropic nystagmus at the Dix Hallpike and/or Semont maneuver. In the remaining 3 patients right horizontal canal was interested with a position geotropic paroxysmal bilateral nystagmus more evident on the right side. In these 7 patients the remaining vestibular clinical tests were negative. All the 7 patients were efficacy treated by liberatory maneuvers. Three patients referred acute objective vertigo associated to neuro-vegetative symptoms (emesis) and they had a possible diagnosis of right vestibular neuritis showing: spontaneous horizontal left beating nystagmus, harmonic alteration of the vestibule-spinal test (Romberg test, Fukuda test, star shaped march test, index finger test), positivity of HIT, HST and vibratory test. These patients were treated with corticosteroids therapy with an improvement of the vertigo in 4-5 days. A complete resolution of dizziness and

vertigo was obtained in one month (central vestibular compensation).

Finally, one patient referred a sudden right hearing loss with tinnitus, acute objective vertigo and neuro-vegetative symptoms (emesis). Audiological tests showed a total right deafness with normal tympanograms and absent acoustic-stapedius reflexes stimulating right ear. She also showed a spontaneous horizontal left beating nystagmus, harmonic alteration of the vestibulo-spinal test (Romberg test, Fukuda test, star shaped march test, index finger test), positivity of HIT, HST and vibratory test. In this woman we also performed caloric test demonstrating right vestibular areflexia. This patient also showed a secondary positional vertigo associated to positional nystagmus at the Semont maneuver related to a Lindsay-Hemenway syndrome (7).

DISCUSSION

Vertigo is frequently experienced during pregnancy and it is the most common complaint by pregnant women to primary care. In a prospective study by Schmidt et al. (8) the authors discovered that 52% of their pregnant cohort complained of vertigo, considering different manifestations of vestibular disorders, such as unbalance, gait deviations, gait instability, a feeling of floating, rotation and falls. These disorders affect the life routine; family, social and professional relations; cause loss of self-confidence, concentration and performance, concentration and work, causing frustration and depression (9).

This high percentage and our results indicate that vertigo in pregnancy must be carefully investigated. Unfortunately, the question concerning the incidence of these pathologies in pregnant patients remains open and often this symptom is underestimated, leaving patients without any treatment because of the difficult diagnostic and therapeutic management during the pregnancy period.

First of all, it must be considered that it's advisable to avoid radiological diagnostic tests (for example the brain CT) in these patients. Therefore the clinical vestibular evaluation takes on considerable importance both for the diagnosis

of an otoneurological pathology and for the exclusion diagnosis.

In the current study we showed that vestibular system can be involved in the etiology of the symptoms. At the admission of patients detailed clinical history must be carefully considered in order to address the diagnostic management of patients. In our patients we found that vertigo has been described as objective spontaneous or positional, associated to emesis in all cases. These findings led to the indication to perform a clinical oto-neurological examination.

On the other hand results of vestibular evaluation showed in seven patients a BPPV and in four a vestibular neuritis, confirming the vestibular involvement.

BPPV in particular is a most frequent cause of vertigo in general population accounting for 20-40% of all cases, with a reported incidence of 0.6% per year, a prevalence between 10.7 and 64.0 cases per 100,000 population and a lifetime prevalence of 2.4% (10-11). Pathophysiology of the disease is still debated, however the role of different pathologies can be hypothesized. Also our study group has recently demonstrated that comorbidity is strictly related to the recurrence of BPPV (12). The role of vascular involvement has been extensively studied and confirmed by different authors (13-14). Also female sex represent a factor risk for BPPV with a pronounced female preponderance (6.8:1 female to male ratio) in BPPV in the teenage group (15) probably due to hormonal conditions (16). In our series of BPPV pregnant women we can hypothesize both a vascular and hormonal involvement in 5/7 patients. In the last two patients only hormonal factors could be considered. Some studies describe the association between dizziness, tinnitus and sudden hearing loss due to hearing and balance alterations with the action of estrogen and progesterone on the cochlea, posterior labyrinth and central auditory pathways (17-18-19-20).

In females, any change in the metabolism of steroid hormones (estrogen and progesterone), responsible for the ovarian cycle can cause complications, among them we list vestibular alterations. These alterations may be peripheral or central and they may occur not only during gestation, but also during the normal menstrual cycle, during menopause and during the pre-menstrual time (21).

In all cases of vestibular neuritis we have described, we can hypothesize a vascular etiology as suggested by the history of previous TIA, Heart disease and Migraine with aura in one patient; by the migraine present in another woman and by the thrombophilia (discovered just for vertigo) in another patient.

In literature is widely described that pregnancy is characterized by a high-flow and low-resistance state with progressive cardiovascular accommodations that persist in the postpartum period (22). Furthermore, it is assumed that estrogen induces vascular supply to the macula and otoconia due to varied glucose and lipid metabolism (23). In current literature, this peculiar topic has been investigated by few studies and the number of presented cases is very limited. Çoban et al. described four pregnant women diagnosed with BPPV during their gestational periods; the authors discussed the role of the hormonal instabilities and alterations as a cause of BPPV in this group of patients. Another possible cause for BPPV in pregnant women they presented is the prolonged bed rest. Moreover, three of the four patients described, were diagnosed during the late gestational weeks, when sleeping and daily activities are usually restricted. They also suppose the usual attitude to sleep on their left sides as another risk factor for BPPV in pregnant women (24).

Recently, there have been reports that calcium and vitamin D metabolism disorders are risk factors for BPPV. Calcium and vitamin D metabolism is usually affected in pregnancy, especially in the late trimesters due to the rapid growth of the fetus. This may be another risk factor for pregnant women suffering from BPPV (25).

A limitation of our study is the number of patients included (11 cases) who cannot be representative of pregnancy-related risk population. In addition, different kind of vertigo with an heterogeneous pathogenesis are included in the same group. About the diagnostic and therapeutic procedures, some maneuvers in pregnancy may be performed but the age of the pregnancy may represent a potential restriction for some types of maneuvers (26). It would be advisable to implement multidisciplinary dedicated protocols in which each specialist should play a specific role in the clinical diagnostic and therapeutic strategy.

Although our case series is small, our results are

interesting and indicate the need to evaluate very carefully a vertigo during pregnancy, in all cases. Moreover comorbidity must be investigated because they often represent an important risk factor for vertigo. About the pathogenic mechanism, the vascular etiology, strictly related to the pregnancy hormonal variations, is often hypothesized, therefore the fundamental role of the screening for thrombophilia and the subsequent anticoagulant therapy is confirmed. So it's fundamental a multidisciplinary approach that involves otolaryngologist, neurologist and gynecologist both for diagnostic purposes and, about the therapy, for the possible toxicity and teratogenicity of some drugs. Finally, increasing the interest in this topic, a clearer definition about the roles of different diagnostic tool and specialist could be provided in order to define specific clinical protocols.

CONCLUSION

In conclusion, there are many otologic and neurotologic manifestations during pregnancy. Although most of them are benign and transient and sometimes they revert to normality in postpartum, the otolaryngologist should acknowledge these conditions and their appropriate management and treatment. Otolaryngologists should be familiar with the potential effects that pregnancy can determine on the vestibular system to be able to reassure the patient with an informed consultation. On the other hand, also gynecologists should be familiar with the otoneurological involvement in the pregnancy. Cooperation between gynecologists, neurologist and ENT specialists is strongly advisable for the choice of the appropriate rehabilitative and/or pharmacological treatment and it's essential to an optimal prognosis. In fact, an incorrect understanding of the therapeutic options often determines a suboptimal treatment because of the hesitation to prescribe any medication to pregnant women for the possible toxicity and teratogenicity, although many drugs can be considered safe, according to the current evidence.

CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

Table I. Clinical features of patients included in the study

Patient	Age	Gestational Weeks	Symptoms	PTA (dB)	Tympanogram	Acoustic-stapedius	Vestibular Evaluation	Comorbidity	Diagnosis	Neurological evaluation	Therapy
1	31	36	Positional Vertigo, Emesis	15	A-A	Normal	Positional Ny (Semont M)	None	PSC-BPPV (right)	Clinically negative	Rehabilitative
2	32	14	Positional Vertigo, Emesis	18	A-A	Normal	Positional Ny (Dix Hallpike M)	Gestosis	PSC-BPPV (left)	Clinically negative	Rehabilitative
3	33	12	Positional Vertigo, Emesis	10	A-A	Normal	Positional Ny (Dix Hallpike M)	AV Block	PSC-BPPV (left)	Clinically negative	Rehabilitative
4	30	35	Positional Vertigo, Emesis	15	A-A	Normal	Positional Ny (Semont M)	Diabetes and hypertension	PSC-BPPV (right)	Clinically negative	Rehabilitative
5	34	23	Positional Vertigo, Emesis	10	A-A	Normal	Geotropic Position Ny (Pagnini M)	Gestosis	HSC-BPPV (right)	Clinically negative	Rehabilitative
6	35	28	Positional Vertigo, Emesis	10	A-A	Normal	Geotropic Position Ny (Pagnini M)	Not	HSC-BPPV (right)	Clinically negative	Rehabilitative
7	33	25	Acute vertigo, Emesis	10	A-A	Normal	Spontaneous Horizontal Ny left beating	-Previous TIA, -Migraine with aura, -Heart disease (aneurysm of the interatrial septum with mitral valve prolapse and mild tricuspid dysplasia)	Vestibular right neuritis	Clinically negative MRI negative	Corticosteroids
8	38	16	Acute vertigo, Emesis	15	A-A	Normal	Spontaneous Horizontal Ny left beating	-Migraine, -Cellac disease, -Onset after progesterone intake for amniocentesis	Vestibular right neuritis	Clinically negative MRI negative	Corticosteroids
9	42	30	Acute vertigo, Emesis, monolateral deafness and tinnitus	Total right deafness; 10 left	A-A	Absent right; normal left	Spontaneous Horizontal Ny left beating (right vestibular areflexia); positional Ny (Semont M)	Thrombophilia with increased D-dimer	Right labyrinthopathy, Lindsay-Hemenway	Clinically negative MRI negative	Corticosteroids Anticoagulants (Heparin)
10	34	27	Acute vertigo, Emesis	15	A-A	Normal	Spontaneous Horizontal Ny left beating	Not	Vestibular right neuritis	Clinically negative, Little altered signal intensity area (4 mm) at MRI	Corticosteroids
11	30	6	Positional vertigo, Emesis, Headache	10	A-A	Normal	Geotropic Position Ny (Pagnini M)	-Migraine -Recurrent Abortions: treatment with antiaggregants	HSC-BPPV (right)	Clinically negative	Rehabilitative

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Ovarian cancer surgery and BRCA test: a nationwide Italian survey

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ABSTRACT

Background. Ovarian cancer (OC) is the most lethal gynecological malignancy in developed countries. Beyond surgery, the backbone treatment of advanced OC is platinum-based chemotherapy. The traditional treatments can be improved by the addition of new target therapies and the breast-related cancer antigens (BRCA) genes represents a potential therapeutic target. Current guidelines recommend BRCA testing for all epithelial OC patients. The objective of the present study is to elucidate the actual scenario of the Italian OC care centers regarding surgery and BRCA testing.

Methods. We conducted a web-based cross-sectional national survey. All invited participants received an e-mail with a 21-item electronic questionnaire accessible through a direct anonymized link. No formal statistical hypothesis was predefined according to the exploratory intent of the survey.

Results. Two hundred-sixtythree potential centers were involved in the survey; 109/263 centers (41.4%) declared advanced OC treatment expertise and are more frequently located in Northern and Central Italian regions ($p=0.0003$). In the southern Italy, OC centers usually refer patients to other centers ($p=0.005$). Most centers (>50%) perform BRCA test in more than 60% of their OC patients but only 36.1% of centers request BRCA status on tumor tissue (sBRCA).

Conclusions. BRCA testing is not homogeneously diffused throughout Italian regions and overall sBRCA testing is not high (36.1%). In the era of personalized medicine, sBRCA testing should be offered to all epithelial OC patients to guarantee target therapy and prevention strategies for relatives with BRCA mutation.

Key words: ovarian cancer; surgery; BRCA test; survey

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SOMMARIO

Obiettivo. Il carcinoma ovarico è la prima causa di morte tra le neoplasie ginecologiche nei paesi sviluppati. Oltre alla chirurgia, il trattamento standard del carcinoma ovarico avanzato è rappresentato dalla chemioterapia a base di platino. L'aggiunta di nuove terapie target, come i farmaci che agiscono sui geni BRCA, possono migliorare l'efficacia delle terapie tradizionali. Le linee guida attuali raccomandano l'esecuzione del test BRCA per tutte le pazienti con tumore ovarico epiteliale.

L'obiettivo del presente studio è quello di chiarire l'effettivo scenario dei centri di cura del carcinoma ovarico avanzato in Italia, per quanto concerne la chirurgia e l'approccio di richiesta del test BRCA.

Metodi. Abbiamo condotto un'indagine nazionale trasversale online. Tutti i partecipanti invitati hanno ricevuto un'e-mail con un questionario elettronico di 21 domande, accessibile tramite un collegamento diretto anonimo. Nessuna ipotesi statistica formale era predefinita in accordo con l'intento esplorativo dell'indagine.

Risultati. Duecentosessantatre centri potenziali sono stati coinvolti nell'indagine; 109/263 centri (41,4%) hanno dichiarato di trattare il tumore ovarico avanzato; questi si trovano più frequentemente nelle regioni dell'Italia settentrionale e centrale ($p=0.0003$). Nell'Italia meridionale, i centri di solito indirizzano i pazienti ad altri centri ($p=0.005$). La maggior parte dei centri (50%) esegue il test BRCA in più del 60% delle pazienti con tumore ovarico, ma solo il 36,1% dei centri richiede il test BRCA sul tessuto tumorale (sBRCA).

Conclusioni. Il test BRCA non è diffuso in modo omogeneo in tutte le regioni italiane e i test sBRCA complessivi non sono elevati (36,1%). Nell'era della medicina personalizzata, il test sBRCA dovrebbe essere offerto a tutte le pazienti con tumore ovarico epiteliale per garantire strategie terapeutiche e di prevenzione per i parenti affetti da mutazione BRCA.

INTRODUCTION

Ovarian cancer (OC) is the most lethal gynecological malignancy in developed countries (1). It is the seventh most common cancer in women worldwide, accounting for nearly 4% of all new female cancer cases (2). According to data from AIOM and AIRTUM, nearly 5,200 new OCs were diagnosed in 2018 in Italy (3). Approximately 90% of all OC cases are epithelial (4). High grade serous ovarian cancer (HGSOC) is the most common subtype, often diagnosed in Stage III (51%) and IV (29%), when disease has already spread beyond the peritoneum leading to a modest 5-year-cause specific survival of 42% and 26%, respectively (5). Standard front-line treatment for advanced OC has remained cytoreductive surgery with the goal of no residual disease (R0), followed by the combination of platinum and taxane chemotherapy with the addition of bevacizumab in first line treatment of “high risk” patients (6).

In case of recurrence/relapse the platinum free interval (PFI) has been used up to now to guide therapeutic choices. Nowadays the definition of PFI results outdated considering the new emerging therapies (target and not target) (7).

In the era of tailored medicine, the study of biological features and molecular pathways in OC identified other factors responsible for treatment response, overcoming the traditional dichotomy: platinum-sensitive vs platinum-resistant patients (8). In this scenario, breast related cancer antigens (BRCA) and homologous recombination deficiency (HRD) status can be considered as novel biomarkers predictive of response to standard chemotherapy (platinum agents, pegylated liposomal doxorubicin and trabectedin) as well as to poly-adenosine di-phosphate (ADP) ribose polymerase (PARPs) inhibitors (PARPi) treatment. HGSOC are characterized by ubiquitous TP53 mutations, and significant focal DNA copy number alterations (9). Approximately 15–20% of HGSOCs may be inherited, with the most common germline mutations related to alterations in BRCA1 and BRCA2 genes. In absence of a germline mutation, the somatic mutation rate reported in available literature ranges between 5% to 7% and frequency as well as type of mutations differs among populations (10). When either BRCA1 or BRCA2 is defective, homologous recombination is dysfunctional and

the reparation of Double-Strand-Break (DSBs) is performed through alternative repair mechanisms such as nonhomologous end-joining (NHEJ) and single-strand repair (SSBs) (11). SSBs repair involves a variety of mechanisms such as base excision repair (BER) and nucleotide excision repair, all of which are supported by PARPs proteins (12). PARPs constitute a family of 18 proteins involved in SSBs and BER, which are activated by DNA damage and facilitate DNA repair. PARP inhibitors prevent the repair of DNA SSBs, transforming them into DNA DSBs. When homologous recombination is not efficacious (HRD), as it is in patients with BRCA mutations, the DNA DSBs cannot be repaired and the PARP inhibition ultimately results in cell death. This mechanism, named synthetic lethality, is an important therapeutic target in HGSOC (13). Therefore, we designed a national survey across Italian centers/institutions with the aim to collect data regarding practices in OC surgery. Secondly, we defined the current scenario of BRCA testing at the time of diagnosis to improve awareness of target therapies in advanced OC patients. The survey was carried out by SIGO (Società Italiana di Ginecologia e Ostetricia).

MATERIALS AND METHODS

Survey Development

We collected data on routine clinical practice in the management of advanced OC and BRCA testing with a questionnaire-based survey, which we designed with a panel of experts including physicians, statisticians, and data managers. A subset of physicians, not directly involved in the survey development, validated the questionnaire regarding readability, usability, and clarity of questions and were asked to describe drawbacks as well as suggestions for improvements. Details about center location, type of center/institution, number and features of surgery, number of BRCA tests performed annually have been collected. The final survey contained 21 questions (**Figure 1**). The institutional e-mail addresses of potential participants were retrieved from the health ministry's database containing all Italian gynecologic units. The survey was emailed to national centers including

1 Do you perform ovarian cancer surgery?
2 How many primary debulking surgery (PDS)/year?
3 How many interval debulking surgery (IDS)/year?
4 Which is your optimal cytoreduction rate (Residual tumor=0) in PDS?
5 Which is your optimal cytoreduction rate (Residual tumor=0) in IDS?
6 Do you have a multidisciplinary tumor board (MTD)?
7 Which other physician are involved in MTB?
8 Which is the timing of MTB?
9 Is the intraoperative histological examination a matter of practice?
10 How many days pass between surgery and definitive histological diagnosis?
11 What is the rate of performing BRCA test in high grade serous ovarian cancer patients?
12 Do you perform somatic BRCA test?
13 If yes, which is the rate of somatic BRCA test?
14 Which is the medical specialist who requests somatic BRCA test?
15 Which physicians provide pre BRCA test counselling?
16 Do you perform pre BRCA test counselling at the same timing of surgery informed consent?
17 What is the timing required to obtain BRCA somatic test results?
18 Do you perform germline BRCA test in case of a positive somatic BRCA test?
19 If so, which is medical specialist who requests germinal BRCA test?
20 Do you offer gBRCA test to patient's family members in case of gBRCA positive results?
21 If so, which physician requests BRCA test?

Figure 1. Survey 21 items questionnaire

community hospitals and academic institutions as an online-available questionnaire. A dedicated electronic Case Report Form (eCRF) was created to collect data. Study data was accrued prospectively and managed using REDCap electronic data capture tools hosted at the

Fondazione Policlinico Universitario A. Gemelli IRCCS (<https://redcap-irccs.policlinicogemelli.it/>). REDCap (Research Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing an intuitive interface for validated data entry, automated export procedures for seamless data downloads to common statistical packages and procedures to import data from external sources (14).

Only people officially registered for this survey obtained a user login to access the REDCap web platform and entered/managed data. The questionnaire structure and format allowed the direct capture of data into Redcap database amenable to be subsequently used for statistical analysis. The survey was anonymous. All participants were invited to respond the 21 items questionnaire that assessed physicians' practice about ovarian cancer surgery (10 items) and BRCA testing attitude (11 items) as reported in Figure 1. Responders did not receive any remuneration. After the first invitation, if no response was obtained after 15 days, two further reminders were sent.

Statistical Analysis

Results are presented as absolute frequency (percentage). Centers' characteristics were described referring to whole Italy and were additionally stratified for three geographical areas: North, Center and South; Italian islands were included in the last group. χ^2 or Fisher's exact tests were used to compare characteristics of centers belonging to different geographical areas. Two-sided tests were applied and the significance level was set at $p < 0.05$. All statistical calculations were performed using the Stata software version 13.0 (Stata Corp, College Station, TX).

RESULTS

Geographic Area, Practice Settings

This survey was conducted from June 2018 to September 2018. **Table I** and **Table II** summarize OC surgery and BRCA testing center characteristics according to geographical areas with corresponding questions.

Table I. Hospital characteristics according to geographical areas *

Characteristic	All centers	Northern Italy	Central Italy	Southern Italy and Island	p
Centers involved in the survey					
Centers participating to the survey	263	107	47	109	
Centers surgically treating advanced ovarian cancer	109/263 (41.4)	60/107 (56.1)	17/47 (36.2)	32/109 (29.4)	0.0003
Centers which address patients to referral centers	133/138 (96.4)	42/45 (93.3)	27/29 (93.1)	64/64 (100)	0.05
Primary Debulking Surgery (PDS)					
Nr of surgeries per year					0.23
0-20	58/109 (53.2)	30/60 (50.0)	12/17 (70.6)	16/32 (50.0)	
20-50	42/109 (38.5)	27/60 (45.0)	4/17 (23.5)	11/32 (34.4)	
>50	9/109 (8.3)	3/60 (5.0)	1/17 (5.9)	5/32 (15.6)	
Percentage of optimal cytoreduction					0.65
< 50%	14/108 (13.0)	7/60 (11.7)	1/17 (5.9)	6/31 (19.4)	
50-70%	38/108 (35.2)	23/60 (38.3)	5/17 (29.4)	10/31 (32.3)	
>70%	56/108 (51.9)	30/60 (50.0)	11/17 (64.7)	15/31 (48.4)	
Interval Debulking Surgery (IDS)					
Nr of surgeries per year					0.42
0-20	85/109 (78.0)	49/60 (81.7)	14/17 (82.4)	22/32 (68.7)	
20-50	20/109 (18.3)	10/60 (16.7)	2/17 (11.8)	8/32 (25.0)	
>50	4/109 (3.7)	1/60 (1.7)	1/17 (5.9)	2/32 (6.3)	
Percentage of optimal cytoreduction					0.24
< 50%	10/107 (9.3)	8/59 (13.6)	0/17 (0.0)	2/31 (6.4)	
50-70%	27/107 (25.2)	11/59 (18.6)	5/17 (29.4)	11/31 (35.5)	
>70%	70/107 (65.4)	40/59 (67.8)	12/17 (70.6)	18/31 (58.1)	
Multidisciplinary Tumor Board (MTB)					
Nr of centers with MTB	94/109 (86.2)	55/60 (91.7)	15/17 (88.2)	24/32 (75.0)	0.09
People involved					
Gynecol-oncologist	94/94 (100)	55/55 (100)	15/15 (100)	24/24 (100)	-
Oncologist	91/94 (96.8)	54/55 (98.2)	14/15 (93.3)	23/24 (95.8)	0.37
Pathologist	82/94 (87.2)	51/55 (92.7)	12/15 (80.0)	19/24 (79.2)	0.11
Radiotherapist	74/94 (78.7)	46/55 (83.6)	13/15 (86.7)	15/24 (62.5)	0.10
Radiologist	64/94 (68.1)	41/55 (74.5)	11/15 (73.3)	12/24 (50.0)	0.09
Surgeon	52/94 (55.3)	30/55 (54.5)	11/15 (73.3)	11/24 (45.8)	0.24
Anesthesiologist	16/94 (17.0)	10/55 (18.2)	2/15 (13.3)	4/24 (16.7)	1.00
Other	12/94 (12.8)	8/55 (14.5)	1/15 (6.7)	3/24 (12.5)	0.83
Meeting frequency					
Weekly	43/90 (47.8)	30/54 (55.6)	5/15 (33.3)	8/21 (38.1)	0.19
Twice monthly	31/90 (34.4)	16/54 (29.6)	8/15 (53.3)	7/21 (33.3)	0.23
Monthly	16/90 (17.8)	8/54 (14.8)	2/15 (13.3)	6/21 (28.6)	0.33
Histology					
Routinary histological examination during surgery	86/108 (79.6)	49/60 (81.7)	13/17 (76.5)	24/31 (77.4)	0.84
Time from surgery to histological results					0.002
< 30 days	99/108 (91.7)	59/60 (98.3)	16/17 (94.1)	24/31 (77.4)	
≥ 30 days	9/108 (8.3)	1/60 (1.7)	1/17 (5.9)	7/31 (22.6)	

Results are presented as n (%). * According to National Institute of Statistic (INSTAT) classification.

Table II. Survey characteristics related to BRCA according to geographical areas *

Characteristic	All centers	Northern Italy	Central Italy	Southern Italy and Island	p
Percentage of HGSOV patients tested for BRCA mutation per center					
0-30%	31/106 (29.2)	16/58 (27.6)	6/17 (35.3)	9/31 (29.0)	
31-60%	15/106 (14.2)	7/58 (12.1)	2/17 (11.8)	6/31 (19.4)	
> 60%	60/106 (56.6)	35/58 (60.3)	9/17 (52.9)	16/31 (51.6)	
sBRCA					
Nr of centers which performed sBRCA	39/108 (36.1)	19/60 (31.7)	7/17 (41.2)	13/31 (41.9)	0.56
Percentage of sBRCA performed per center					0.57
0-30%	19/38 (50.0)	7/18 (38.9)	5/7 (71.4)	7/13 (53.8)	
31-60%	4/38 (10.5)	2/18 (11.1)	1/7 (14.3)	1/13 (7.7)	
> 60%	15/38 (39.5)	9/18 (50.0)	1/7 (14.3)	5/13 (38.5)	
Specialist involved in sBRCA management					
Test request					
Gynecol-oncologist	33/66 (50.0)	16/40 (40.0)	7/13 (53.8)	10/13 (76.9)	0.07
Oncologist	34/66 (51.5)	18/40 (45.0)	7/13 (53.8)	9/13 (69.2)	0.31
Pathologist	4/66 (6.1)	3/40 (7.5)	0/13 (0.0)	1/13 (7.7)	0.82
Genetist	11/66 (16.7)	6/40 (15)	4/13 (30.8)	1/13 (7.7)	0.09
Pre-test counseling					
Gynecol-oncologist	38/66 (57.6)	23/40 (57.5)	6/13 (46.2)	9/13 (69.2)	0.49
Oncologist	32/66 (48.5)	18/40 (45.0)	7/13 (53.8)	7/13 (53.8)	0.78
Pathologist	0/66 (0.0)	0/40 (0.0)	0/13 (0.0)	0/13 (0.0)	-
Genetist	13/66 (19.7)	7/40 (17.5)	4/13 (30.8)	2/13 (15.4)	0.68
Concomitant sBRCA and surgical informed consent					
Time to obtain result					
< 2 months	23/47 (48.9)	11/24 (45.8)	3/10 (30.0)	9/13 (69.2)	1.00
2-3 months	17/47 (36.2)	8/24 (33.3)	5/10 (50.0)	4/13 (30.8)	0.16
> 3 months	7/47 (14.9)	5/24 (20.8)	2/10 (20.0)	0/13 (0)	0.21
germline BRCA					
Nr of gBRCA test performed in case of sBRCA mutation	39/47 (83.0)	20/23 (87.0)	9/12 (75.0)	10/12 (83.3)	0.67
Specialist involved in test request					
Gynecol-oncologist	26/60 (43.3)	16/39 (41.0)	5/11 (45.5)	5/10 (50.0)	0.87
Oncologist	28/60 (46.7)	16/39 (41.0)	5/11 (45.5)	7/10 (70.0)	0.26
Pathologist	3/60 (5.0)	2/39 (5.1)	0/11 (0.0)	1/10 (10.0)	0.58
Genetist	17/60 (28.3)	11/39 (28.2)	4/11 (36.4)	2/10 (20.0)	0.71
Nr of gBRCA requested for relatives in case of patient's gBRCA mutation					
Specialist involved in test request for relatives					
Gynecol-oncologist	21/58 (36.2)	12/37 (32.4)	4/12 (33.3)	5/9 (55.6)	0.42
Oncologist	23/58 (39.7)	12/37 (32.4)	4/12 (33.3)	7/9 (77.8)	0.39
Pathologist	2/58 (3.4)	1/37 (2.7)	0/12 (0.0)	1/9 (11.1)	0.35
Genetist	28/58 (48.3)	20/37 (54.1)	7/12 (58.3)	1/9 (11.1)	0.05

Results are presented as n (%). HGSOV: High Grade Serous Ovarian Carcinoma. BRCA: Breast Related Cancer Antigen, sBRCA: somatic BRCA. gBRCA: germline BRCA. * According to National Institute of Statistic (INSTAT) classification

Two hundred- sixtythree potential centers were involved in the survey, 109/263 (41.4%) reported an advanced OC treatment expertise and compiled questionnaire; the remaining 154 centers declared no expertise in surgical OC treatment and therefore, did not answer further survey questions.

133/138 centers non treating advanced OC (96.4%) address patients to referral centers, 5/138 (3.6%) declared they haven't a referral center, while 16/154 (10.4%) centers didn't answer the question.

58/109 (53.2%) centers treat with primary debulking surgery 0-20 advanced OC patients/year, 42/109 (38.5%) centers treat 20-50 OC patients/year and only 9/109 (8.3%) treat more than 50 patients/year (third question). 51.9% (56/108) of centers affirmed to achieve an optimal cytoreduction in more than 70% of cases (fourth question).

According to the fifth question about number/year of interval debulking surgery (IDS), 85/109 (78%) centers treat 0-20 advanced OC patients/year, 20/109 (18.3%) centers treat 20-50 OC patients/year, 4/109 (3.7%) treat more than 50 patients/year.

In this clinical setting, 65.4% (70/107) of centers declare to perform an optimal cytoreduction in more than 70% of cases (sixth question).

94/109 (86.2%) centers have a multidisciplinary tumor board (MTB): gynecology-oncologist, oncologist and pathologist are the most frequent involved physicians. In most cases (47.8%) the MTB meeting frequency is weekly, in 34.4% twice monthly in 17.8% monthly. Four centers declare to have MTB only on request. Almost all centers (99/108, 91.7%) perform histological examination in less than 30 days from surgery and 86/108 (79.6%) routinely use an intraoperative frozen section evaluation.

The remaining 11 items deal with physicians' attitude of BRCA testing in OC patients.

About the half of the centers perform a BRCA test in more than 60% of their HGSOE patients but only 36.1% of centers request sBRCA. Usually, sBRCA testing is requested by gynecology-oncologists who performed correspondingly pre-test counselling. Less than half of the centers obtain sBRCA consent at the same time of surgical informed consent. The time needed to obtain BRCA test results takes from 2 to more than 3 months in about 50% of centers. In case of

sBRCA mutation, 83% (39/47) of centers perform gBRCA (on blood sample) to verify the constitutional nature of mutation. More than 90% of centers involved in this survey, request BRCA testing for relatives in case of patients' gBRCA mutation. Geneticists (48.3%) or oncologist (39.7%) usually are involved in gBRCA test requests for patients' relatives.

Regarding centers geographical distribution, 60/107 (56.1%) of centers are located in Northern Italy, 17/47 (36.2%) are located in the center of Italy and the remaining 32/109 (29.4%) were distributed across South of Italy and the Islands, as summarized in **Table I**. Centers more specialized in advanced OC surgery are likely located in North and Central Italy ($p=0.0003$). All centers in South Italy usually address patients to other referral centers ($p=0.05$).

No other statistically significant associations were found in this survey.

DISCUSSION

To the best of our knowledge, this is the first national survey about common practices and beliefs regarding BRCA testing among Italian physicians trained in OC care.

Inhomogeneity of referral centers' geographical distribution corresponds to organizational heterogeneity of regional health systems. Yet, the Southern OC centers, usually address patients in Northern and Central referral centers ($p=0.05$). The standard OC surgery (removal of the adnexa, uterus, omentum, and pelvic and para-aortic lymph nodes) is often associated with complex surgical techniques used to debulk advanced disease like bowel resection, splenectomy, partial liver resection, peritoneal or diaphragmatic stripping. In this regards, high-volume hospitals report statistical significant survival benefits (15). Different factors are associated with improved survival for OC patients. In particular, centralized primary care and complete cytoreduction rate at primary surgery are two of the strongest predictors to survival (16,17).

Moreover, complete cytoreduction rate is improved by centralization together with shortened time interval from surgery to chemotherapy, which may impact survival outcome. OC surgery centralization and a larger proportion of

patients achieving an optimal cytoreductive surgery emphasize the importance of experienced and skilled surgeons.

Regarding MTB, more than 80% of the centers declared to meet at least twice a month. A recent systematic literature review (which included 27 articles) reported that MTBs have impact on management decisions of cancer patients. In fact, between 4% and 45% of OC cases discussed in MTBs experienced changes in diagnostic reports. Additionally, patients discussed at MTBs were more likely to receive more accurate pre-operative staging and neoadjuvant/adjuvant therapy (18). The review showed limited evidence of survival outcomes, in contrast to earlier large cohort studies (19).

Regarding OC molecular characterization, sBRCA testing is uncommonly prescribed by Italian physicians; in fact only 36.1% of centers request sBRCA. In 2019, implementation of BRCA testing in OC patients and their relatives was updated (20). The sBRCA test can identify variants acquired as somatic mutations in addition to constitutional defects. Hence, in the event of a positive result, the BRCA variant must be verified with peripheral blood to verify its constitutional origin. The somatic analysis enables physicians to identify a fraction of around 7% of OC patients with a pathogenic BRCA variant that would remain unknown if test would be restricted to peripheral blood analysis (21). The complexity of the BRCA test in terms of interpretation require laboratories with high expertise to ensure high quality data. In the United States, BRCA testing has become universal for all OC patients over the last few years. It has been estimated that medical and surgical risk reduction strategies, applied to BRCA positive healthy family members, could decrease the ovarian cancer incidence by 40% within 10 years (22). Taking into account that effective OC prevention and/or screening methods are not available, it is extremely important to offer BRCA test to HGSOc. The importance of BRCA test at diagnosis, preferably on tissue, is underlined by the availability of target therapy as Parp-inhibitors. In the first-line Solo 1 trial in advanced OC, Olaparib significantly improved progression-free survival in BRCA mutated patients; the risk of disease progression or death was 70% lower with Olaparib than with placebo (23). This landmark trial has changed practice for BRCA

mutated (somatic or germline) OC patients.

Even though the present investigation is innovative and analyzes such an intriguing and interesting aspect of OC diagnostics and treatments, our study had several limitations. Firstly, this survey, might be subject to selection bias that could arise from the recruitment of a specific group of physicians that responded in a specific way to our questions. Nevertheless, we consider the physician sample as representative for the OC Italian Leads of the major Gynecologic Units (Unità Operative Complesse). This list was available from Italian Ministry of Health. Moreover, this survey was conducted from June 2018 to September 2018, before publication of SOLO1 trial results. The impressive SOLO1 results could modify physician attitude to request BRCA testing due to remarkable results of the trial. Finally, according to the exploratory intent of the survey, we did not predefine any formal statistical hypothesis and the purpose of the survey was to explore this topic more thoroughly to develop some specific hypothesis or predictions that can be tested in future research.

In our experience, Italian physicians involved in OC patient surgery do not prescribe sBRCA test as routine of patient journey. This survey is the starting point to capture the current OC patient access to BRCA molecular testing. This survey can be useful to collaborate with both institutions and patient associations to implement the OC molecular diagnostic pathway across Italian regions. In particular, referral centers should set up biobanks and national bioinformatics database to share patient's data in order to implement molecular diagnostics to ensure target therapies. Furthermore, the Italian Centers could collaborate to develop uniform molecular diagnostic test method and result reports.

Standardizing BRCA testing at diagnosis, as per international guidelines suggestion, could ensure that patients are correctly treated. sBRCA testing should be offered to all epithelial OC patient to enhance the availability of target therapy (Parp-I) and to improve, in case of a BRCA constitutional variant, relatives' prevention strategies.

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CONFLICTS OF INTERESTS

AP worked in the AstraZeneca Medical Department until December 2018.

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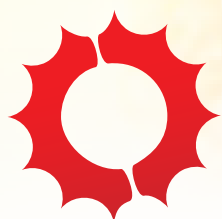
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