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Miscarriage: a social networks users' point of view

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ABSTRACT

Background. Every day several posts are published on social networks, however there are limited evidences about how miscarriage is perceived and disclosed by social network users. The aim of our review was, therefore, to collect studies focused on posts published in the main social networks (Instagram, Facebook, and Twitter) and regarding miscarriage, in order to give an overview of feelings and emotions perceived by social network users after a miscarriage.

Methods. In October-November 2020, we searched in the electronic database PubMed (MEDLINE), all the peer-reviewed journal articles published between January 2000 and November 2020. We considered eligible articles that reported clear data on 1) analyzed social network; 2) number of posts; 3) year of study; 4) interesting data about emotions perceived by social network users about miscarriage. Studies should be written in English, French, Spanish, Italian.

Results. Of the 32 found publications, 2 met the inclusion criteria: the studies have been conducted between 2017-2019 and analyzed posts published on Instagram and Twitter. Up to 50.6% (Twitter) and 16.2% (Instagram) women expressed grief and anger or annoyance in their posts but also conflicted emotions, regret, hope. In particular, the experience of recurrent pregnancy loss was perceived by women as stressing for their future pregnancies. Some women decided to show their miscarriage on the social network considering this action as an attempt to both seek support and to offer solidarity to others. Celebrity disclosures, published researches about miscarriage risk factors, and/or preventive methods for miscarriage generated a high number of discussions on Twitter.

SOMMARIO

Background. Diversi post vengono pubblicati ogni giorno sui social network, tuttavia ci sono prove limitate su come l'aborto spontaneo venga percepito e condiviso dagli utenti dei social network. Lo scopo della nostra review è stato, quindi, raccogliere studi incentrati sui post pubblicati nei principali social network (Instagram, Facebook e Twitter) riguardanti l'aborto spontaneo al fine di avere una panoramica delle sensazioni e delle emozioni percepite dagli utenti dei social network dopo un aborto spontaneo.

Metodi. Nel periodo ottobre-novembre 2020 abbiamo cercato nei database elettronici PubMed e MEDLINE tutti gli articoli di riviste peer-reviewed, pubblicati tra gennaio 2000 e novembre 2020. Abbiamo considerato idonei per la review gli articoli che riportavano dati chiari su: 1) social network analizzati; 2) numero di post; 3) anno dello studio; 4) dati interessanti riguardanti le emozioni percepite dagli utenti dei social network sull'aborto spontaneo. Abbiamo considerato solo gli studi in inglese, francese, spagnolo, italiano.

Risultati. Delle 32 pubblicazioni trovate, 2 soddisfacevano i criteri di inclusione: gli studi sono stati condotti nel periodo 2017-2019 e hanno analizzato i post pubblicati su Instagram e Twitter. Fino al 50.6% (Twitter) e al 16.2% (Instagram) delle donne hanno espresso dolore, rabbia o fastidio nei loro post, ma anche emozioni contrastanti, rimpianti, speranza. In particolare, l'esperienza di aborti ricorrenti è stata percepita dalle donne come stressante per le loro future gravidanze. Alcune donne hanno deciso di condividere la loro esperienza di aborto sui social network considerando questa azione come un tentativo sia di cercare sostegno sia di offrire solidarietà

Conclusions. Miscarriage is usually perceived in a negative way on social networks, however, participation in online media could be useful because of the support usually given by others that experienced the same event. However, both studies that we included in the review didn't allow a complete overview about this interesting topic.

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agli altri. Rivelazioni pubbliche da parte di celebrità, ricerche pubblicate sui fattori di rischio dell'aborto spontaneo e/o metodi di prevenzione dell'aborto spontaneo hanno generato un numero elevato di discussioni su Twitter.

Conclusioni. L'aborto è solitamente percepito in modo negativo sui social network, tuttavia, la partecipazione nei social network potrebbe essere utile a causa del supporto solitamente fornito da altri che hanno vissuto lo stesso avvenimento. Tuttavia, entrambi gli studi che abbiamo incluso nella review non hanno consentito di ottenere una panoramica completa su questo interessante argomento.

Key words:

Miscarriage; social network; Instagram; Twitter; Facebook.

INTRODUCTION

In western societies, it has been estimated that approximately 20% of pregnancies end in spontaneous abortions (also known as miscarriages) within the first 22 weeks (1). Miscarriage has an important psychological impact on women's life (2). Women who experience miscarriage have an increased risk of depression and anxiety in the first month following Early Pregnancy Loss (EPL). Male partners also experience depression and anxiety, albeit at a generally lower level. There is also evidence of post-traumatic stress symptoms relating to EPL (2, 3).

Women with recurrent miscarriage show psychological distress higher after abortion, it persists even after one year and with the worst outcomes in women from rural areas (4). A comparative study on quality of life among women with and without recurrent miscarriage using the 36-Item Short Form Survey (SF-36) and the Hospital Anxiety and Depression Scale (HADS) demonstrated that women with recurrent miscarriage reported extensive functional disability, and lower level of well-being compared to women without recurrent miscarriage (5). Results from another study indicated that anxiety, depression, and perinatal grief can persist for a long period after miscarriage (6). Also, more attention should be given to women in vulnerable situations, such as immigrant women, women with low socioeconomic status, or childless women. Beyond those personal and contextual factors, the quality of the

conjugal relationship and the level of satisfaction with health care appeared to be important protective factors against mental health problems after a spontaneous abortion (6). In couples with recurrent pregnancy loss (RPL), medical professionals need to take a holistic and couple-focused approach in their treatment including attention to the psychological impact and cumulative effect of the multiple RPL on the couple (7). Research also shows that women with pregnancy loss are dissatisfied with the medical care they receive and wish for more information, empathy, psychological support and follow-up, and to know the cause of the pregnancy loss (8). Social networks can play a crucial role in helping and encouraging individuals after pregnancy loss by providing a positive support system that helps in lessening feelings of grief and loss (9). On the other side, previous studies evidenced a "dark side" of some social networks, especially those based mostly on photos/videos, demonstrating a significant relationship between more social network activity based on sharing, seeing, and commenting on photographs and body image impairment and problems in regulating emotions (10). However, the role of these image-based social media platforms in health communication and public health remains underexplored (11, 12).

This narrative review tries to provide a descriptive overview of miscarriage as perceived and described via posts published on Instagram, Facebook, and Twitter. We summarize the research literature up

to November 2020. The scope of this review is a priori limited to Instagram, Facebook and Twitter because considered the most used social networks (13, 14). The aim of our review was, therefore, to collect studies focused on posts published in the main social networks (Instagram, Facebook, and Twitter) regarding miscarriage to give an overview of feelings and emotions perceived by social network users after a miscarriage.

MATERIALS AND METHODS

Search strategy

In October-November 2020, we searched in the electronic database PubMed (MEDLINE), all the peer-reviewed journal articles published between January 2000 and November 2020. This narrative review aimed to identify and describe the messages presented in posts published in the main social media (Twitter, Instagram, and Facebook) about miscarriage to identify future directions for research, surveillance, and regulation. The following combination of MeSH terms was used “Social Network AND Miscarriage”, “Facebook AND Miscarriage”, “Twitter AND Miscarriage”, “Instagram AND Miscarriage” OR Pregnancy Loss, Spontaneous abortion.

Inclusion criteria

We considered eligible articles (original articles, but also letters to the editor if containing original data) that reported clear data on 1) analyzed social network; 2) number of posts; 3) year of study; 4) interesting data about emotions perceived by social network users about miscarriage. We considered eligible studies written in English, French, Spanish, Italian.

The studies we decided to include were descriptive studies in which authors extracted posts containing phrases or hashtags related to miscarriage, and then analyzed them using especially a qualitative method.

Study selection and data extraction

Studies were selected in a 2-stage process. Titles and abstracts were screened independently by two authors (MC, GT). The same authors independently assessed studies for inclusion and extracted data. A manual search of references within the included

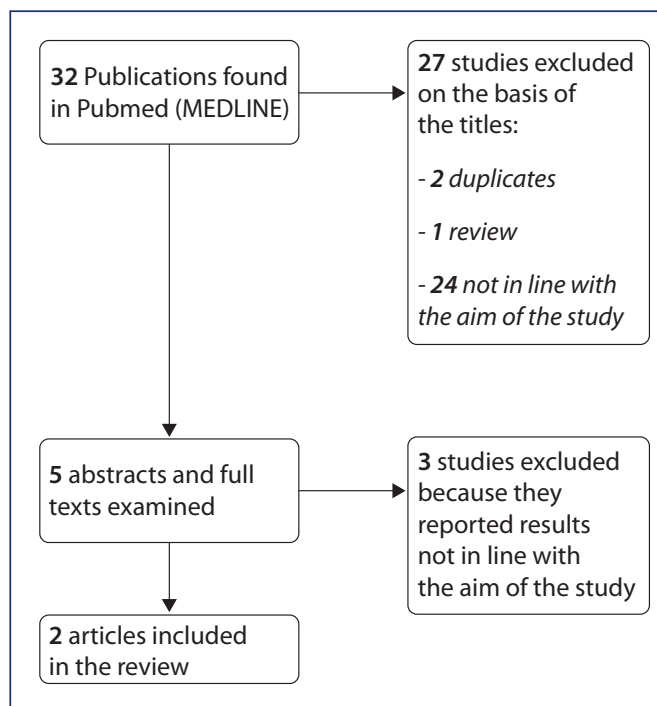


Figure 1. Flow diagram for identifying studies to be included in the review.

studies was also performed to avoid any missing relevant data. Any disagreement concerning the extracted data was resolved by consensus. Selected articles were read in full by MC and GT.

RESULTS

The literature search yielded 32 publications. The titles of these manuscripts were screened, resulting in 5 studies considered potentially eligible to be included in the review (2 articles were excluded because they were duplicates, 1 was a review, 24 were not in line with the aim of the study). 3 studies were excluded after an examination of the abstracts and the full texts (they reported results not in line with the aim of the study). Finally, after this exclusion, we identified 2 manuscripts (15, 16) (figure 1).

Main characteristics of the studies

The principal results of our review are shown in table I. The only two studies included in our review have been published in 2019 and 2020 and have been conducted between 2017-2019. In these studies, authors collected and analyzed posts retrieved from 2 social networks: Instagram and Twitter (respectively 200 posts and 291443 tweets). No article about Facebook has met the inclusion criteria.

Table I. Selected characteristics of the studies included in our review (n/r = not reported; * = year of publication; 1 = number of users from whom the posts have been analyzed; 2 = datum calculated on 7282 tweets from 5079 users).

| AUTHOR, YEAR* | SOCIAL NETWORK | YEAR | STUDY DESIGN | POSTS (N) | USERS (1) | MAIN FINDINGS |
|---------------|----------------|-----------|-----------------|-----------|-----------|--|
| Cesare, 2019 | Twitter | 2017-2018 | n/r | 291443 | 138658 | <ul style="list-style-type: none"> · The major topics of discussion were: Michelle Obama (8.4%), Celebrity (23.0%), Preterm birth (10.9%), Politics (17.6%), Loss and Anxiety (10.1%), Ectopic pregnancy (7.5%), Healthcare (10.7%), Influenza Vaccine (11.7%). · Increase of discussions about miscarriage during celebrity disclosures, research studies on risk factors and preventive methods for miscarriage. · Michelle Obama's disclosures generated 3051 tweets (3.6 times the magnitude of the highest weekly average). · 50.6% and 16.2% expressed grief and anger or annoyance respectively (2). · 0.6% expressed relief because not ready to be parent. · 14.2% of tweets were neutral. · 4.7% of tweets blamed their body, behavior of circumstances for their miscarriage. |
| Mercier, 2020 | Instagram | 2019 | Cross sectional | 200 | n/r | <ul style="list-style-type: none"> · A lot of posts reported a rich description of medical and physical experience of miscarriage. · A lot of posts reported a description of lack of fetal cardiac activity. · 2 posts described the experience of miscarriage on an airplane and in a bathroom. · Miscarriage is often seen as a milestone. · A lot of posts are a request of support. · A lot of posts reported fear or conflicted feelings about sharing one's miscarriage. · Most experienced feelings: grief, conflicted emotions, anger, regret, hope. · Recurrent pregnancy loss has effects on one's identity, and creates worry and stress about a future pregnancy. · Some posts reported the term "Rainbow baby" used to describe a baby born after a miscarriage. · Remembrance of the miscarriage is seen as a common element of coping. |

Miscarriage: Social networks users' point of view

Our analysis has shown that negative feelings are often strictly connected to miscarriage and emerge also in social networks: up to 50.6% (Twitter) and 16.2% (Instagram) women expressed grief and anger or annoyance in their posts but also conflicted emotions, regret, hope. In some cases, women blamed their body, behavior of circumstances for their miscarriage. In particular, the experience of recurrent pregnancy loss was perceived by women as stressing for their future pregnancies. Only a small percentage (0.6%) of women expressed relief because declared not to be ready to be parents. Some women decided to show their miscarriage on the social network considering this action as an attempt to both seek support and to offer solidarity to others. An important public figure, *i.e.*, Michelle Obama, publicly talked about her experience of miscarriage; this news increased in an important way the average number of tweets. Beyond celebrity disclosures, a general increase of discussions

on Twitter has also been observed in occasion of published researches about miscarriage risk factors, and /or preventive methods for miscarriage.

DISCUSSION

Several posts are published every day on social networks, however there are limited evidences about how miscarriage is perceived and disclosed by social network users. Our review tried to analyzed the published articles on this topic in order to have an overview.

Our results, although limited to two social networks (Instagram and Twitter), show that the general attitude related to miscarriage and disclosure on social networks post is generally negative. In this context, previous studies suggested that women (especially those with recurrent spontaneous abortions – RSA) should receive psychological counseling to handle the distress they experience. Thus, the psychological management of distress in

women with miscarriage must be included in the treatment of RSA (4). In fact, compassionate care, informational guidance, and timed follow-up positively impact patient outcomes (17).

Education of healthcare professionals is important to address misconceptions about miscarriage, increase confidence in providing support, and promote effective care. Women and families experiencing miscarriage need privacy and timeliness in care. Interventions such as funded midwifery loss care or a routine telephone follow-up call could improve access to care and help healthcare professionals ensure that families obtain the type and amount of support that they need (1, 18).

On Instagram, the experience of recurrent pregnancy loss was perceived by women as stressing for their future pregnancies. It is well described in the literature that women with recurrent miscarriage tend to have a lower level of well-being compared to women without recurrent miscarriage, and an appropriate treatment is essential for their psychological distress (5). Our review highlighted a general increase of discussions in occasion of public disclosures, published researches about miscarriage risk factors, and/or preventive methods for miscarriage. Michelle Obama revealed she suffered a miscarriage 20 years ago and used IVF to help conceive her daughters Sasha and Malia (19). The public disclosure of a health problem from a public figure is an extensively studied phenomenon. For example, Angelina Jolie, Jade Goody, Kylie Minogue, Nancy Reagan, and Steve Jobs were studied for their cancers (breast, cervical, pancreatic, *etc.*) (20-22). Lots of studies highlighted a clear effect of celebrity medical news on the population (for example the openness to consider genetic testing as an early detection tool for women with a family history of breast and/or ovarian cancer) (23).

Although a very high percentage of women expressed grief, anger, and other negative feelings after a miscarriage, some women decided to show their miscarriage on the social network considering this action as an attempt to both seek support and to offer solidarity to others. Many individuals ex-

pressed their solidarity on social networks and sympathized with persons affected by some pathologies (24). It is also evident that people affected by some pathologies interact with one another via social networks. As social media become frequently used as a source of medical information, professionals should be aware of the content available and consider using them as a means to provide health education (24). As described by other authors, for example, one of the disadvantages of participating in some forums was becoming emotionally affected by other members' negative experiences. Further, some participants described that some information given in the forums were inaccurate. Forum members can have limited knowledge of the medical history of the other members and therefore the given advice and information may not be adequate in every case (25).

Both studies that we included in the review didn't allow a complete overview about this interesting topic so we encourage other colleagues to perform further studies – in other countries, or including a higher number of posts or analyzing other social networks such as Facebook – in order to have a better knowledge about it.

CONCLUSIONS

Social networks are interesting tools, well studied for their influence on an extensive number of people (26). Miscarriage is usually perceived in a negative way on social networks, however, participation in online media could be useful because of the support usually given by others that experienced the same event. Solidarity, receiving and giving support are certainly positive elements, however, they should be integrated by an emotional (and professional) support given by healthcare workers.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Bacillus Calmette-Guerin (BCG) in immunotherapy of ovarian tumors: from the beginning to recent findings. May it still be relevant?

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ABSTRACT

Bacillus Calmette-Guerin (BCG) is still the most used form of vaccination against virulent *Mycobacterium tuberculosis* (Mt) from 1921. Inactivated Mt is capable to induce an immune response principally on T1 lymphocytes in experimental animal (Freund complete adjuvant) and in men (BCG). In 1970 it was thought that inducing this response by intradermal or intratumoral BCG might have been useful in the treatment of advanced ovarian carcinoma. This study was published in 1974. In 1976 intracavitary BCG was first applied in the treatment of superficial bladder tumors; in 1990 its use was accepted by the U.S. Food and Drug Administration and still now it represents the golden therapy for this kind of tumor. Recently a derived BCG cell wall skeleton (SW-BCG) preparation was used intracutaneously in ovarian cancer patients after surgical removal of tumors with lymphadenectomy. It was seen that lymphadenectomy causes a significantly reduced survival as a sign of a decreased immune response. Although BCG use in human tumors, like bladder cancer, is still relevant, many other important discoveries of inhibitors of immunity cellular checkpoint (CTLA-4, PD-1) in human immunotherapy have been successfully done in the last years. Nevertheless BCG, which was used first, has represented and still represents a model for cancer immunotherapy and its mechanism of action is not fully understood: BCG might act stimulating the immune response of T lymphocytes mobilization, modifying the TA expression, removing the immune defense blocking

SOMMARIO

Il Bacillus Calmette-Guerin (BCG) è la forma di vaccinazione più utilizzata nell'uomo contro la tubercolosi da *Mycobacterium* (Mt) dal 1921 ai giorni nostri. Il Mt inattivato è in grado di indurre una risposta immunitaria principalmente sui linfociti T1 negli animali da esperimento (come adiuvante completo di Freund) e negli uomini (BCG). Nel 1970 si è pensato che, provocando questa risposta con l'uso di BCG intradermico (*i.d.*) o intratumorale, ciò avrebbe potuto essere utile anche nei tumori solidi umani, in particolare nel trattamento di stadi avanzati di carcinoma ovarico e nello studio della reattività alla tubercolina o agli antigeni dello stesso tumore. I risultati di questa indagine, eseguita nella Clinica Ostetrica e Ginecologica dell'Università di Padova sono stati pubblicati negli Atti del 56° Congresso SIGO, nel 1974. Nel 1976 il BCG è stato poi proposto con trattamento intracavitario per la prima volta nel carcinoma della vescica superficiale e nel 1990 il suo uso è stato accettato dalla Food and Drug Administration (USA) e ancora oggi rappresenta la terapia prescelta in questo tipo di tumore vescicale. Recentemente la preparazione della parete cellulare del BCG (SW-BCG) è stata utilizzata per via sottocutanea per testare l'effetto della linfoadenectomia nel carcinoma ovarico: è stata osservata altresì una significativa correlazione della linfoadenectomia con una ridotta sopravvivenza delle pazienti. Benché l'uso del BCG in alcuni tumori, come il cancro alla vescica, sia ancora attuale, sono stati fatti molti altri importanti progressi, soprattutto riguardanti inibitori dei checkpoint cellulari

and making TA recognizable by more stimulated CD-8T and NK cells. BCG might be successfully associated to anti PD-1 or CTLA-4 cancer immunotherapy in non-responding patients, since they have different mechanisms of action, to give a more active and potent immune response.

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INTRODUCTION

Bacillus Calmette Guerin (BCG) is a bovine inactivated and not virulent tubercular bacillus used for years to immunize against the diffusion of tuberculosis due to the infection of virulent *Mycobacterium tuberculosis* (Mt) and firstly administered in human beings in 1921.

BCG is the only vaccine against tuberculosis. It is the most widely administered vaccine and usually a part of the routine newborn immunization schedule. BCG vaccine also offers protection against non-tuberculous mycobacteria infection like leprosy and Buruli ulcer. BCG vaccine is safe and is not associated with severe complications (1).

Only in the last fifty years, starting from 1970, it was proved that BCG vaccine does not only protect against tuberculosis, but its use also produces benefits against other bacterial diseases (2), decreases the incidence of allergic diseases (3) and is useful in the treatment of certain malignancies (4, 5). Despite its limitations, to date in bladder cancer it has not been surpassed by any other treatment (6). An evolution from topical BCG intravesical administration, which was first approved by the U.S. Food and Drug Administration in 1990, to PD-1/PD-L1 immunotherapy has been recently reviewed (7).

The aim of this review in the light of other experiences, is to look at a possible role of BCG or its

dell'immunità (CTLA-4, PD-1), e l'immunoterapia è stata eseguita con successo in molti tipi di tumori umani negli ultimi anni. Tuttavia, il BCG ha rappresentato e rappresenta ancora un modello di indagine per la reattività immunitaria e il suo meccanismo d'azione non è del tutto compreso: il BCG potrebbe agire stimolando la risposta immunitaria dei linfociti T, modificando l'espressione degli antigeni tumorali (AT), rimuovendo alcuni blocchi immunodifensivi e rendendo l'AT più riconoscibile dalle cellule CD-8T e NK così stimulate. In studi recenti si ritiene che il BCG potrebbe essere associato con successo agli inibitori dei checkpoint immunitari PD-1 e CTLA-4 nei pazienti che non rispondono o che hanno delle recidive dopo tali terapie.

Key words:

Bacillus Calmette-Guerin (BCG); human immunotherapy; ovarian tumors; others malignancies; tumor's T-lymphocytes response.

derivatives in cancer immunotherapy, in particular in ovarian cancer where it was firstly proposed, in addition to immunotherapies with anti-checkpoint inhibitors.

BCG use in human solid tumors

Ovarian tumors

Use of BCG in solid malignancies started with ovarian tumors in 1970 in the Obstetric and Gynaecological Clinic of the Padua University where 19 patients with advanced ovarian carcinoma were tested with the intent of improving the lymphocytes response against cancer by intradermal or intratumoral BCG injections (8-10). In these patients BCG was injected in 16 patients weekly by repeated intradermal injections (indirect administration) and in 3 cases by direct intratumoral injection (direct intraparenchymal injections). In two of these patients BCG was injected into subcutaneous metastatic nodules after having boosted them twice intradermally; in the third, BCG was injected in the proper tumor parenchyma inside the peritoneal cavity.

The initial approach was to attack the ovarian tumor using BCG intradermally or in the site of the same tumor thus initiating, eliciting, and improving an immunogenic response to tumor similarly to what observed in experimental animals with the use of Freund complete adjuvant (which contains

also killed Mt in a mineral oil solution). This would have happened through a modification of surface tumor antigen presentation and recognizing, and activating lymphocytes T-cell responses, lymphocytic infiltration into tumors and cell death. In fact, cell-wall skeleton (CWS) fraction was found to be the major adjuvant-active principle of mycobacterial cells which were used in Freund's complete adjuvant (FCA). It was then suggested that dendritic cells and macrophages express two sorts of receptors, Toll-like receptors, TLR-2 and TLR-4, and a putative binding receptor for BCG-CWS, whose signaling pathways lead to a sufficient antigen-presenting state in the activation of the innate immune system (11, 12).

Intradermal BCG (indirect) and intradermal tumor homogenate testing reaction

16 patients of variable age, from 28 y. to 62 y. were studied. All with gynaecological malignancies (8 ovaric, 7 cervical carcinoma, 1 endometrial). All these patients had been previously treated surgically or by radiant therapy, judged in an advanced stage of the disease and no longer treatable. They were preliminary tested with intradermal (*i.d.*) injection of 0.1 ml tuberculin (Mantoux reaction) and 14 were found negative and 2 weakly positive (-+). The tumor homogenate was prepared as reported by Kaham B.D. (13). BCG vaccination was done by intradermal injections or by grating dermal scarification. After the first BCG vaccination 14/16 patients had a response to BCG vaccination, 8 weak (1 +), 4 mild (++) and 2 strong (+++). Repeated vaccinations (two to three every week) with 0.1 ml dose determined a very strong reaction in 13 patients; two on which BCG was used form dermal scarification (30 mg bacterial patina each) had high temperature (39-40 °C) for one day and vomit.

It was then thought that it would be interesting to test the skin anti-tumor homogenate in the patients in which dermal reaction to BCG was found improved: in other words, to see if the skin reaction against BCG might have elicited also some reaction against the homogenate preparation of tumor cells of the same women. All these patients were found, before initiating BCG, preliminary negative to *i.d.* injection of their own tumors.

Only in two patients, which had been BCG negative and then BCG positive (one cervical carcinoma and one ovarian bilateral Krukenberg tumor), the tumor homogenate determined an intradermal reaction, the first with a diameter of 8 mm after 24-48

hours, interpreted as delayed hypersensitivity reaction to tumor); in the other patient after 48 hours an infiltrate erythematous (classified ++-) was present. These cases were the first preliminary experimental evidence that in solid gynaecological advanced tumors in all the 19 patients admitted to the study there was an absent or weak (less than one plus), tuberculin *i.d.* reaction.

This reaction can be changed to a very strong one after repeated (2 to 3) vaccinations with BCG.

This delayed hypersensitivity reaction obviously acts primarily against BCG but also, in few cases (2/16) in our experience, against its own tumor antigen homogenate showing a delayed hypersensitivity cancer *i.d.* reaction (DIX) evident after 48 hours, when this DIX was absent before the BCG vaccination.

Thus, BCG repeated intradermal vaccinations may indirectly improve also an anti-tumor response in a minority of previously non-responding patients. This observation may have some importance and take some place for the proposal of supporting with BCG *i.d.* boosting other kind of immunotherapies when there is some relapse or loss of efficacy of the immunotherapy chosen primarily.

Intratumoral (i.t.) BCG injection (direct)

These studies have been a direct consequence of the first 16 reported in point 1 and are briefly described here:

- a. in the first case (F.N. 48 y.) (at TIII N1 stage) 30 mg. of BCG were injected at open abdomen just in thickness of main tumor mass in the omentum. Metastases were observed in the intestine and in the peritoneum. The carcinoma was histologically judged of probable intestinal origin. BCG showed no apparent regression of the tumor and the patient deceased apirectic due to abdominal and pulmonary carcinomatosis after 5 months.
- b. In the second case (S.M.30 y.), with an initial totally absent tuberculin reaction, BCG was injected *i.d.* at weekly intervals with 30 mg of bacterial patina (b.p.) each time such determining a very intense local reaction (+++). It was then decided to make the BCG injection directly in two parasternal visible metastatic nodules, with the aim of addressing the lymphocytes reaction against the tumor cells: this was done twice, at a distance of a week, the first with 6 mg, the second with 15 mg of b. p. After 4 hours of the 2nd injection followed a rise in temperature (up to 40 °C) that decreased in the afternoon (**figure 1**). This young women was at almost ter-

minimal stage with pleural bilateral effusion and bone metastasis radiologically diagnosed of an advanced granulous ovarian carcinoma and weighted 35 Kg After having left the hospital, this patient regained 10 Kg of weight and her general conditions improved in an unexpected way; her life was prolonged of one year. Then tumor relapsed after she refused other advised boost treatment with BCG.

- c. In the third patient (D.B.G., 57 y.), not judged susceptible to any known therapy, BCG was directly injected into the parenchyma of an advanced great ovarian tumor with peritoneal metastasis (stage T III) only once at a dose of 30 mg, after opening the abdominal wall to visualize the peritoneal cavity and the actual state of the tumor with an explorative laparotomy. The body temperature of this patient in the days after the BCG intratumoral injection is shown in the reported figure (figure 2). No chemotherapy was used. This patient left the Hospital after 48 days with an apparent complete recovery and the

clinical disappearance of the tumor, which was before seen and histologically demonstrated. This patient survived 10 years, there has been no return of the tumor and she died of heart attack. At my knowledge, these cases represent the first reports that BCG influences, changes or improves the clinical behavior in human solid tumors – gynaecological in the described ones – most probably after having changed the immune response against them. This reaction has been obtained with direct intratumor injections of visible parasternal nodules done after a previous *i.d.* boosting in the patient (S.M.) and with a single injection of 30 mg of BCG inside the tumor’s parenchyma, in a very advanced case of ovarian cancer with multiple peritoneal metastasis, in the other patient (D.B.G.). This approach led to complete and definitive recovery of patient D.B.G., who survived tumor-free for 10 years without any further chemotherapy or surgery, and to an unexpected one year survival in the other patient (S.M.) with visible clinical improving and a 10 kg. weight gain. In the last patient

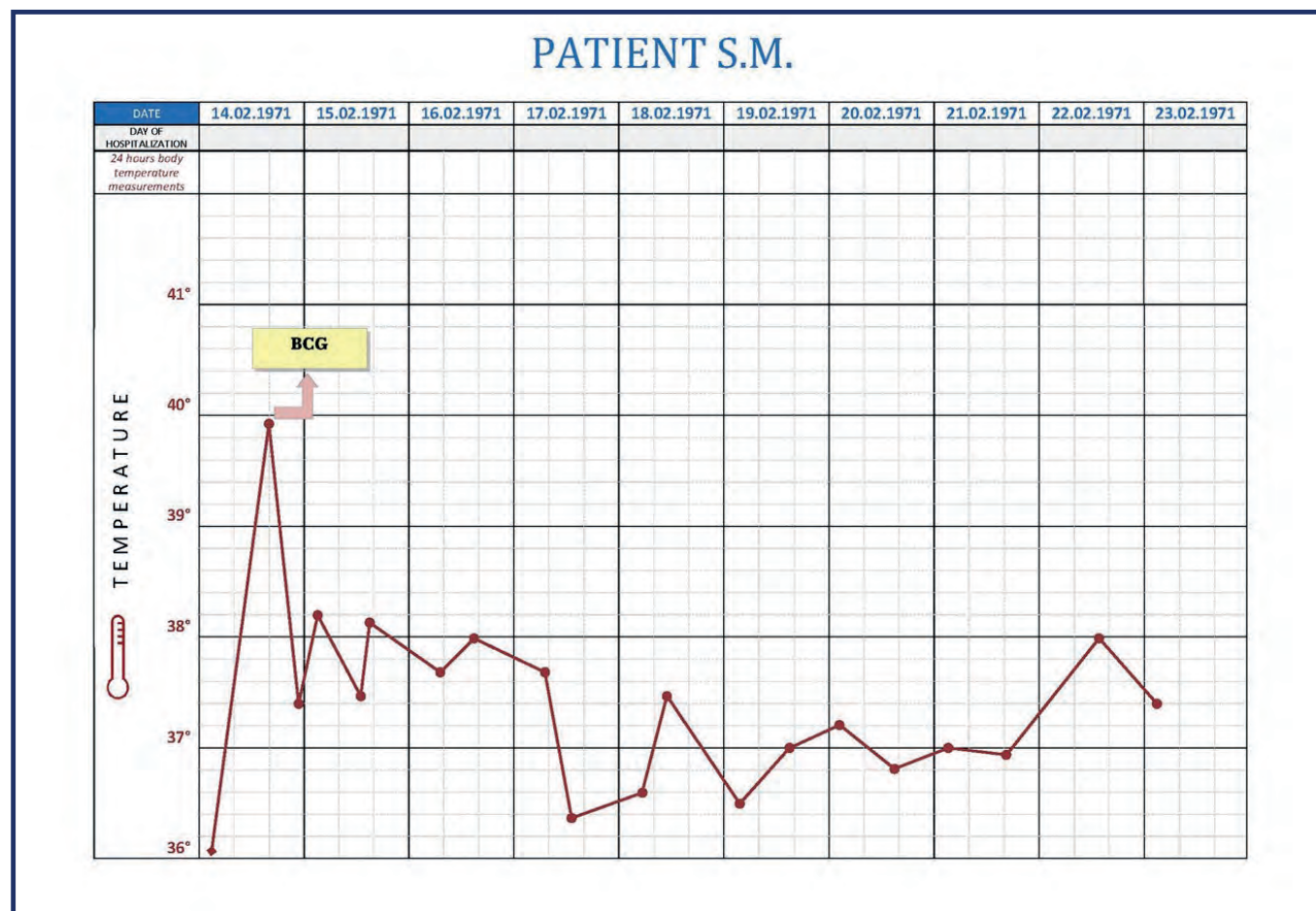


Figure 1. Behavior of body temperature after intralesional administration of BCG in patient S.M. (30 y.).

(F.N.) BCG seemed to have no evident clinical effect and this patient survived 5 months.

Immunotherapy in ovarian cancer

These first reports were confirmed by successive studies: over the course of time the presence of T cells within the epithelial component of tumors (TILs), as histologic evidence of anti-tumor immunity has been associated with a survival advantage. It was also demonstrated that intraepithelial TILs are robust predictors of outcome in ovarian cancer and define a specific class of patients, whose distinct tumor biology should be taken into account in devising appropriate therapeutic strategies.

There are not enough data about BCG used alone in different types of ovarian cancers and in concomitance or in absence of other therapies (surgical, chemotherapy, anti-PD-1, etc.), but since BCG elicits T-cell response, an indirect answer to this question may be found in the reported study on the prognostic significance of tumor infiltrating T-cell (TILs) in ovarian cancer regarding 1815 pa-

tients which is, to my knowledge, the most complete and convincing about the matter.

The characteristic of the study refers to more than 70% of the patients Stage III-IV. The article doesn't concern BCG use, but as a matter of fact, BCG stimulates TILs infiltration. A lack of intraepithelial TILs is significantly associated with a worse survival among patients as shown in the table reported in the same paper which shows the experience of many countries (pooled HR: 2.24, 95% CI; 1.71-2.91) (14).

It was recently proposed a standardized methodology to assess tumor-infiltrating lymphocytes (TILs) in other solid tumors, as Melanoma, Gastrointestinal Tract Carcinomas, Non-Small Cell Lung Carcinoma and Mesothelioma, Endometrial and Ovarian Carcinomas, Squamous Cell Carcinoma of the Head and Neck, Genitourinary Carcinomas, and Primary Brain Tumors (15). Lack of TILs within the tumors represent always a negative factor for the prognosis. It was reported that in 73 patients with ovarian cancer in which, after surgical removal of tumors with lymphadenectomy, a cell skeleton of

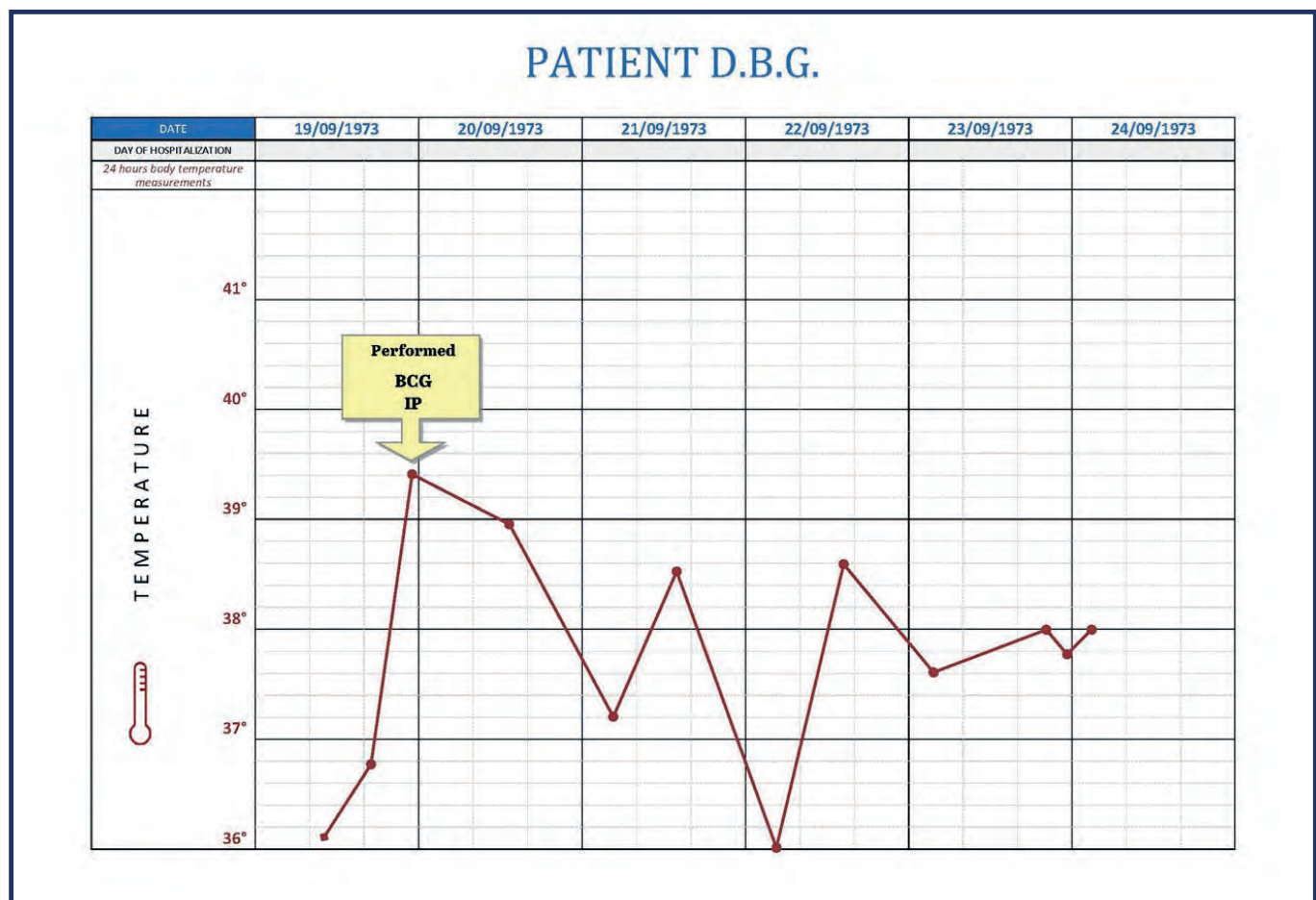


Figure 2. Behavior of body temperature after intralesional injection of BCG in patient D.B.G (57y).

BCG was inoculated intracutaneously, a significantly reduced survival of patients was observed. In view of the importance of the role of lymph nodes in the stimulation of Toll-like receptors by BCG-CWS, it is suggested that lymph nodes should be kept as much as possible to preserve the patient's immunity against cancer (16). Other results suggest that mycobacterial CWS as a universal vaccine vehicle for conjugation of a wide variety of antigens, as Mt derived, constitutes a breakthrough for development of the most promising vaccines for infections, allergic diseases, and cancer (17-19). In 1999 were published the results of multiple immunotherapy with BCG with other adjuvants (Cley's vaccine, mixed bacterial vaccine, transfer factor and lymphoblastic lymphocytes) in 52 patients treated between 1991 and 1997) in different types of cancer at different stages. Apparently this immunotherapy was given not intraparenchymally, not only BCG was used and many patients had undergone other therapies (surgical and others). The cases to be considered "successful" were 4 over 52: Hodking disease, adenocarcinoma of esophageal junction (who received also surgery), a prostate adenocarcinoma who made also the traditional therapy's case of generalized carcinoma of the ovary (20).

Since ovarian cancer is the fifth leading cause of cancer death among women and the most lethal gynaecologic malignancy, enormous progress has been made in the management of this disease. Immune checkpoint inhibitors offer a potential for improved survival. A variety of strategies are possible using new agents and the paper focuses principally on phase III and ongoing trials. New immunotherapeutic approaches based on immune checkpoint inhibitors are currently changing the landscape in melanoma treatment, however, in ovarian cancer, potential success of this therapy relies on better understanding of tumor microenvironment and dominant immunosuppressive pathways, as well as finding reliable biomarkers. So far, described biological drugs and new therapeutic approaches were not shown to cure ovarian cancer, but they bring the long-awaited promise of turning it into a manageable chronic disease (21).

Intraperitoneal, gastric, colon cancer

In 1983 *Corynebacterium parvum* was given intraperitoneally (*i.p.*) in human ovarian carcinoma in 14 cases of advanced ovarian tumors. Administration of *C. parvum i.p.* has augmented the ability of human peritoneal cells to lyse human ovarian car-

cinoma cell lines in the presence of specific rabbit antiserum. In 5 patients out of 11 treatments with *C. parvum* produced surgically confirmed tumor regression (45%) with 2 complete responses (22). Systemic *Corynebacterium parvum* has limited activity in gynaecologic malignancies. Although intraperitoneal *C. parvum* is active, its toxicity is prohibitive. Intraperitoneal alpha-interferon is an active second line agent for minimal residual disease following combination chemotherapy. Intraperitoneal interferon trials are ongoing. The presence of intratumoral T cells independently correlated with delayed recurrence or delayed death in multivariate analysis was associated with increased expression of interferon-gamma, interleukin-2, and lymphocyte-attracting chemokines and with best survival in tumor patients. The presence of intratumor T cells correlates with improved clinical outcome in advanced gastric and ovarian carcinoma. (23-25).

The use of BCG is aimed at improving the T lymphocytes presence in the context of the tumor. BCG responsiveness is believed to be linked to a large number of tumor infiltrating lymphocytes, not only ovarian but also gastric cancer. Surgery reception before BCG may ameliorate the prognosis. A total of 156 patients with stage III or IV gastric cancer who had undergone curative resection were randomly assigned to three treatment groups: BCG + FAM (immunotherapy), FAM (chemotherapy), and control (surgery only). In patients with pT2/T3 primary tumors, 10-year survival was 55.3% for BCG + FAM vs 28.2% for FAM ($P < 0.01$) and 14.6% for the control group ($P < 0.00018$). This study, based on a limited number of patients, indicates that adjuvant immunotherapy (BCG + FAM) may prolong the survival of gastric cancer patients after curative gastrectomy; in particular, in patients with pT2/T3 tumors and intestinal-type primary tumors (26).

Treatment of gastric peritoneal carcinomatosis by combining complete surgical resection of lesions and intraperitoneal immunotherapy using Catumaxomab, a nonhumanized chimeric antibody, is characterized by its unique ability to bind to three different types of cells: tumour cells expressing the epithelial cell adhesion molecule (EpCAM), T lymphocytes (CD3), and also accessory cells Fcγ receptor. Because the peritoneum is an immunocompetent organ and up to 90% of gastric carcinomas express EpCAM, intraperitoneal infusion of Catumaxomab after complete resection of all macroscopic disease (as defined in the treatment of carcinomatosis from colorectal cancer) could therefore

efficiently treat microscopic residual disease (27). *Bacillus Calmette Guérin* (BCG) has a potential anti-tumor effect on gastric cancer, however, the mechanism is still unclear. An experimental study (27) investigated the effect of BCG on gastric cancer cell line MGC-803 and studied the potential cooperation of BCG and lymphocyte in determining the final fate of cancer cells. After treatment with BCG, the cell viability was significantly inhibited in a dosage-dependent manner. Flow cytometry assay showed that apoptosis rates were significantly increased by BCG.

It was found that BCG induced secretion of interferon gamma (IFN γ) from lymphocytes. BCG promotes lymphocyte immunocompetence to induce cell apoptosis and autophagy in MGC-803 cells, inducing the release of IFN γ from peripheral blood lymphocytes (28).

As far as colon cancer is concerned, macropinocytosis has emerged as an important pathway of protein acquisition in cancer cells, particularly in tumors with activated Ras such as pancreatic and colon cancer. Macropinocytosis is also the route of entry of *Bacillus Calmette-Guérin* (BCG) and other microbial therapies of cancer. Using BCG uptake to assay macropinocytosis, we executed a genome-wide shRNA screen for macropinocytosis activators and identified Wnt pathway activation, which is a main regulator of development throughout the animal kingdom, is seen as a strong driver of macropinocytosis (29). In conclusion, it was suggested that the Wnt pathway is a previously unappreciated driver of macropinocytosis in cancer.

With a multicenter, randomized controlled phase III clinical trial in Stages II and III colon cancer, patients with active specific immunotherapy (ASI) using autologous tumor cells with an immunomodulating adjuvant bacillus Calmette-Guérin (BCG) vaccine (OncoVAX) in an adjuvant setting, it was seen that surgery alone cures approximately 65% of Stage II (Dukes B2, B3) colon cancer patients. In the remaining patients, OncoVAX in an adjuvant setting, significantly prolongs recurrence-free interval (57.1% relative risk reduction) and significantly improves 5-year overall survival and recurrence-free survival. This study verified that the use of OncoVAX for patients with Stage II colon cancer not only has significant prognostic benefit and positive clinical outcomes, but also showed that OncoVAX therapy yields impressive health economics benefits (30).

In a very recent report, done using cell wall skeleton of BCG (cws-BCG) in 18 patients with ad-

vanced WT1-expressing cancers refractory to standard anti-cancer therapies (7 melanoma, 5 colorectal, 4 hepatobiliary, 1 ovarian, and 1 lungs), was found a dose-limiting toxicity which occurred in the form of local skin reactions in 2 patients at a dose of 200 μ g although no serious treatment-related systemic AEs were observed. Some patients demonstrated the induction of the CD4 T cell subset and its differentiation from the naïve to memory phenotype, resulting in a tumor response. The RD of BCG-CWS was determined to be 100 μ g/body. This dose was well tolerated and showed promising clinical effects with the induction of an appropriate immune response (31).

Bladder tumors

After 4 decades of use, BCG remains the most effective agent against high risk NMIBC, but it still holds substantial drawbacks. The enduring use of immunotherapy for NMIBC has created a propitious environment to search for better alternatives. There is an increasing number of promising in vitro, animal and early human clinical trials to anticipate a significant therapeutic alternative in the foreseeable future (31). This procedure, in which BCG enters directly in contact with malignant cells was, with the same intent, subsequently applied to bladder cancers with intravesical administration of BCG by intraurethral way of the bladder tumor (published in 1976) (32). In 1990, the U.S. Food and Drug Administration approved the use of intra-vesical BCG for patients with superficial bladder cancer, and this procedure is the recommended standard of care for high-grade non-invasive bladder cancer worldwide (33). The history of BCG and bladder cancer as an immunotherapy of success was then reviewed by Harry and Co (34).

Intravesical therapy with bacillus Calmette-Guérin has proven effects for reducing recurrence, progression, and death from non-muscle-invasive bladder cancer. These advantages are seen mainly when appropriate maintenance therapy is used for 1-3 years, in the context of appropriate patient selection, tumor management, and symptom support for potential side effects (35).

This procedure is still in use and represents the main immunotherapeutic approach in bladder papilloma cancer and is regarded as a golden standard in high and intermediate-risk non muscle invasive bladder cancer. Unfortunately, up to 40% of patients might experience treatment failure. Immune checkpoint inhibitors emerge as new

immunotherapeutics, which in the future might be combined with BCG and may serve as an alternative to radical cystectomy in patients, who failed to respond to BCG alone (37).

At this proposal some points have to be underlined:

1. BCG in bladder cancer works in about the half of the patients;
2. it has effect through direct contact with cancer cells;
3. sometimes the immune response declines with time but can be restored by repeated BCG intravesical boosting;
4. it is not known if an intradermal BCG repeated vaccination might sustain indirectly an anti-cancer direct immunotherapy.

We must then reiterate the conclusion that a certain, though unknown importance should be apparently given to the direct BCG action by intimate contact with tumor cells as well in ovarian as in bladder cancer.

The direct contact of BCG with the tumor cells may change the expression of Tumor Antigens thus exposing a new formed antigen which may elicit the cellular T lymphocyte immune response and the aggression against the tumor in many patients.

Melanoma

Intratumoral immunotherapy has been used in melanoma in which the approach to skin visible tumor is simpler. BCG was used in locoregional immunotherapies and metastasis melanoma (38). Mycobacterium bovis Bacillus Calmette-Guerin (BCG) serves as an adjuvant therapy that induces the recruitment of natural killer NK, CD4⁺, and CD8⁺ T cells and contributes to antitumor immunity. BCG can be administered in combination with chemotherapeutic and immunotherapeutic agents and can be genetically manipulated to produce recombinant BCG (rBCG) strains (39). It has been recently recognized that BCG serves as an adjuvant therapy that induces the recruitment of natural killer NK, CD4⁺, and CD8⁺ T cells and contributes to antitumor immunity. BCG can be administered in combination with chemotherapeutic and immunotherapeutic agents thus increasing the immune response and improving patient survival in melanoma patients. Since early trials BCG based immunotherapy for melanoma consistently showed a trend toward improved clinical outcomes in patients treated with BCG compared with observation alone (40).

Other types of immunotherapy have been trained in this type of tumor. Talimogene laherparepvec

(T-VEC), a genetically modified herpes simplex virus type 1-based oncolytic immunotherapy, is the first oncolytic virus approved by the U.S. Food and Drug Administration for the treatment of unresectable melanoma recurrent after initial surgery and the efficacy and safety data from clinical trials of T-VEC both as monotherapy and in combination with immune checkpoint inhibitors has been proved. Responses were seen in both injected and uninjected lesions including visceral lesions, suggesting a systemic antitumor response. When combined with immune checkpoint inhibitors, T-VEC significantly improved response rates compared with a single agent; similar results were seen with combinations of checkpoint inhibitors and other intratumoral therapies such as CAVATAK, HF10, and TLR9 agonists. Thus, both BCG and T-VEC have been used intratumorally as immunostimulatory agents with the goal of tumor cell lysis, followed by release of tumor-derived antigens and subsequent activation of tumor-specific effector T cells (41).

As far as I know, in melanoma it was never tried to improve a depressed antituberculin immune reaction aspecifically, boosting the patient with intradermal or subcutaneous BCG, before injecting BCG directly in the tumor mass, in association with other chemotherapy or immunotherapeutic approaches.

As a comment, it has to be said that intratumoral immunotherapy can also be done with oncolytic viruses or biology derivative vaccine that change antigen presentation of the tumor as it expected to happen with BCG. The question remains to understand if BCG and oncolytic viruses work with different mechanisms, the former perhaps privileging the immune response, the latter changing the TA presentation to lymphocytes.

Lung cancer

An interesting study has been carried out on the effect of coexistence of concomitant active tuberculosis in patients with non-small cell lung cancer (NSCLC). In fact, concomitant active tuberculosis (TBLC) resembles loco regional immunotherapy of tumor cell vaccine like BCG whose local administration within the lung is not practicable. This study compares the survival and immunological cell profile in TBLC over NSCLC alone and a total of 276 NSCLC were included for a one year period. It was found that the median survival of squamous cell carcinoma (SCC) with an active tuberculosis is significantly longer (p less than 0.01) than adenocarcinoma or undetermined NSCLC with TB. Ac-

tive tuberculosis in SCC increases the expression of CD3, CXCR3 and IP-10. These results show a better survival outcome in NSCLC patients with active tuberculosis. This effect is likely connected with the concomitant presence and perhaps contact of active tuberculosis with the lung cancer cells (42, 43).

In **table I** the types of malignancies with priority BCG treatment and the main reported results are summarized.

Association of BCG with immune checkpoint inhibitors

The release of negative regulators of immune activation (immune checkpoints) that limit antitumor responses has resulted in unprecedented rates of long-lasting tumor responses in patients with a variety of cancers. This can be achieved by antibodies blocking the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) or the programmed cell death 1 (PD-1) pathway, either alone or in combination (44). The main premise for inducing an immune

response is the preexistence of antitumor T cells that were limited by specific immune checkpoints. Most patients who have tumor responses maintain long-lasting disease control, yet one-third of patients relapse. Mechanisms of acquired resistance are currently poorly understood, but evidence points to alterations that converge on the antigen presentation and interferon- γ signaling pathways. New-generation combinatorial therapies may overcome resistance mechanisms to immune checkpoint therapy. The release of negative regulators of immune activation (immune checkpoints) that limit antitumor responses has resulted in an undetectable way (45). A novel dendritic cell immunotherapy with *i.t.* administration of BCG-CWS-treated dendritic cells following tumor cryoablation could be used for the therapy of cancer patients with multiple metastases. But of the most important interest is to underline that the association of BCG with immune checkpoint inhibitors was found to improve in any case the anti-tumor response better than a single agent alone (46). A heat-killed preparation of *Mycobacterium vac-*

Table I. Types of malignancies with priority BCG treatment, references and main results.

| Type | Description | Authors | References | Notes |
|--|---|--|---|--|
| Leukemia | BCG after the graft of the tumor | Mathé G, <i>et al.</i> | Boll Ist Sieroter Milan. 1971 Jul-Aug;50(4):251-9 | Improvement and increase of survival in all children |
| Ovarian cancer | Intraparenchymal tumor BCG injection, without any surgery or chemotherapy at inoperable stage 3-4 | Carretti N, <i>et al.</i> (3 with intralesional BCG, 16 with i.d. stimulation) | Atti del 56° Congresso Nazionale della Società Italiana di Ostetricia e Ginecologia. Padua, 3-5 Oct 1974: pp. 465-8 | 1 complete recovery for 10 years 1 remission with 1 year survival 1 death after 5 months No answer in 16 i.d. treated |
| Bladder tumor | Superficial, non-invasive | Morales A, <i>et al.</i> | J Urol 1976;116: 180-3 | Recommended by FDA in 1990 as standard of care |
| Lung Cancer postop: follow up report | Intrapleural BCG: 5 years follow up reports | Bakker <i>et al.</i> | Cancer Immune Immunother 1986 | 56 p. treated no survival benefits at 2 and 5 years |
| Lung Cancer non-small cells lung cancer post-surgical stage I | Intrapleural BCG | Macchiarini P, <i>et al.</i> | Anticancer Res 1989 | 17 p. no significant improvement at 5 years |
| Intraperitoneal | i.p. alpha 1 interferon Corynebacterium parvum and IL-2 | Foon KA, Fanning; Bast J, <i>et al.</i> | Cancer Research 43,1395-14011983 Semin Surg Onc 1990;6(6):364-8 | Limited activity Prohibitive Toxicity Limited experience |
| Ovarian Carcinoma Stages III-IV | Autologous T lymphoid in vitro activated by i.p. route | Canevari S, <i>et al.</i> | JNCI Oct 1995;87:1463-9 | 23 treated patients Complete response in 3 patients (26, 23, 18 months) |
| Colon cancer stage II | Surgery + Oncovax (BCG) | Uyl-de Groot CA, <i>et al.</i> | Vaccine, 2005 Mar 18;23(17-18):2379-87 | Statistically improves 5 years survival |
| Melanoma | Advanced melanoma | Stewart JH and EA Levine | Expert Rev of Antic Ther 2011; 11:1671-6 | Statistically improves survival compared to patients not treated with BCG |
| Ovarian, Gastric cancer. Regional immunotherapy Systemic review of 5 years | TILs, peripheral blood mononuclear cells | Zeeltman M, <i>et al.</i> | Am J Surg Oct 2016 | Increase of survival in the regional administration of cell-based clinical trials over the last 5 years |

cae (SRL172) has been shown, in recent studies, to be effective in the treatment of adenocarcinoma of the lung and renal cell cancer. It is postulated that the mechanisms of this form of immunotherapy is, at least in part, due to immune regulation, reflected in the selective enhancement of Th1 and down-regulation of Th2 T cell activity (47, 48).

Immunotherapy of the ovarian tumor was reviewed recently. BCG interfering with CTLA-4 and PD-1 tumor's check point inhibitors have demonstrated clinical benefit in several human cancers.

In order to generate effector T cells with ability to recognize tumor *in vivo*, cancer vaccines emerged as an immunotherapeutic approach, but a major question is identifying the most effective and safe vaccine targets in ovarian cancer and the effects of combined therapies (49, 50). Intralesional therapies can provide the necessary stimuli to trigger an innate immune response leading to the production of type I interferons and maturation and activation of dendritic cells that can cross-present tumor antigens to T cells. Intralesional therapies including BCG, viral therapies, inflammatory cytokines may act determining a tumor infiltration with T cells that are directed against tumor antigens leading to the production of type I interferons and maturation and activation of dendritic cells that can cross-present tumor antigens to T cells (51). Recently presented clinical and pre-clinical combination therapies have been developed to overcome resistance and have the potential to substantially extend the therapeutic reach of these revolutionary drugs (52, 53). On the other hand, over the years immunotherapy has become a promising and effective strategy for the treatment of various types of cancer, including ovarian cancer, but despite the clinical success of antibodies against the immune regulators CTLA4 and PD-L1/PD-1, only a subset of people exhibit durable responses suggesting that a broader view of cancer immunity is required (54).

Ovarian cancer is the leading cause of mortality from gynaecological malignancies, and novel treatment modalities, including immune therapy, especially anti-programmed cell death ligand-1/programmed cell death-1 (PD-L1/PD-1) treatment is now focus of attention (55).

Since the presence of tumor infiltrating lymphocytes (TILs) is associated with improved clinical outcome in ovarian cancer patients (14), it was hypothesized that the quality of infiltrating T cells could also be a critical determinant of outcome in ovarian cancer patient. Nevertheless, it must be remembered that a

significant inverse correlation was observed between PD-L1 expression and the intraepithelial CD8⁺ T lymphocyte count, suggesting that PD-L1 on tumor cells directly suppresses antitumor CD8⁺ T cells in ovarian cancer working in an analogous way as anti PD-L1 antibodies initiating the response of cytotoxic T-lymphocyte antigen 4 (CTLA-4) and programmed death receptor 1 (PD-1) (55, 56). In targeting DNA repair pathways of the most genomic unstable cancer, poly-(adenosine diphosphate [ADP])-ribose polymerase inhibitors (PARPi) have been demonstrated as the most effective drug since platinum in high grade serous or endometrioid ovarian cancer. Immunotherapy is strongly pushing the door of ovarian cancer and has the ambition to change the fate of this deadly disease when combined with chemotherapy, vascular endothelial growth factor inhibitor or PARPi. The activity of PARPi could also be improved by modulators of the cell cycle, which are required to give time enough for DNA repair. Even more ambitious are drug targeting the driver p53 mutation or the pathway which inhibit tumor cell apoptosis (57). It was indeed recently found that programmed death-ligand (PD-L1) expression was upregulated in bladder rats cancer cells in response to BCG treatment both *in vitro* and *in vivo*. Moreover, BCG and anti-PD-L1 blockade therapy combination treatment activates a potent antitumor immune response with the increase in the number and activity of tumor-infiltrating CD8⁺ T cells, as well as the reduction in myeloid-derived suppressor cells (MDSCs). This kind of combination immunotherapy elicits prominent tumor growth inhibition and prolonged survival, and was found to be much more effective than either agent alone (58, 59).

The findings highlight the adaptive dynamic regulation of PD-L1 in response to BCG immunotherapy and suggest that BCG and anti PD-L1 combination therapy may be a promising antitumor strategy for improving treatment outcomes in BC as in other tumors.

As a conclusion, BCG immunotherapy might be tried in association with other anti CTLA-4 and PD-1, in tumor relapsing patients.

Concerning surgical immune interventions for solid malignancies, the search yielded 334 relevant publications. There has been an increase in the regional administration of cell-based and viral vector-based clinical trials over the last 5 years. Surgical interventions have been developed for intrapleural, intracranial, intraperitoneal, and intratumoral routes of access to enhance the local de-

livery of these therapies. Multimodality therapies that combine regional immunotherapy with other local and systemic therapies are demonstrating continued growth as the field of immunotherapy continues to expand. Enormous progress has been made in the management of this disease, and new targeted treatments such as antiangiogenic drugs, poly (adenosine diphosphate-ribose) polymerase inhibitors, and immune checkpoint inhibitors offer potential for improved survival (19, 60).

As a summary, may be said that:

1. BCG used intradermally to improve an aspecifically immune reaction provokes only a feeble if any reaction against the tumor (8);
2. BCG as other viral agent (CAVATAK) may determine the complete recovery of the patient and the disappearance of tumor also in non-injected lesions (9, 10, 20, 31);
3. many patients have had other kind of therapies (surgery, chemotherapy) and are seen at different stages of their disease with different histologic characteristics: it is so sometimes difficult to understand if the improvement is caused by BCG or by other concomitant immunotherapy. This paper refers to the use of only BCG in patients in terminal stages of their ovarian cancers and no longer amenable to any other therapy (surgical or chemotherapy (4)).

Antigen-conjugated BCG-CWS vaccine is simple to manufacture, safe, and easy to use. Our results suggest that mycobacterial CWS as a universal vaccine vehicle for conjugation of a wide variety of antigens constitutes a breakthrough for development of the most promising vaccines for infections, allergic diseases, and cancer (61). However, despite all the advances made so far by immunotherapy and combination therapy, the next major challenge involves understanding and overcoming the innate and acquired resistance that prevents most patients from responding to PD-1/PD-L1 blockade. A recent overview of current FDA-approved drugs targeting PD-1 or PD-L1 summarized clinical progress so far (61).

CONCLUSIONS

The approach of intratumoral BCG in the ovarian tumors preceded other successive kind of immunotherapy attempts in other human malignancies as bladder cancer and melanoma and only recently we understood some of its mechanisms of action.

These attempts made in 1970, were the first kind of any human solid cancer immunotherapy, with the reports of 19 ovarian tumors patients, using intratumoral (direct) or intradermal BCG (indirect) injections. They have shown:

- a. the first BCG intraparenchymal injection in the context of a primitive human solid tumor (ovarian) between 1971 and 1973;
- b. the first demonstration that in a very advanced ovarian (TIII) tumor with peritoneal metastasis, complete disappearance of the primitive tumor and peritoneal metastasis were obtained as well as the recovery of the patient and her survival for 10 years, in the absence of any other associated therapy (surgical or chemotherapy);
- c. in other two cases of parasternal subcutaneous metastasis BCG intralesional injection of the tumor was also done with a clear improvement in one terminal stage patient who gained 10 kg of weight and survived one year against any clinical expectation;
- d. the first reports in which it was proposed that a close contact between BCG and tumoral cells may improve the anti-tumor activity immunological answer, perhaps modifying the tumoral antigen presentation to T lymphocytes;
- e. the first observation that there is an absent or weak anti *Mt* dermal reaction in gynecological advanced solid tumors and that this reaction can be changed with *i.d.* BCG repeated injections and becomes strong after 2-3 BCG vaccinations;
- f. the first demonstration that the aspecific *i.d.* BCG administration solicits also, in few patients, a delayed immune response to own tumoral antigen homogenate. This reaction improves the immune aggression of the tumor if the tumor is secondarily injected with BCG, as in the case of patient S.M. The response might be secondarily directed against the same tumor with intralesional injections of BCG or his derivative (BCS-SW) thus can improving, in some patients, the T cells immune response against the tumor.

Successive immunotherapy cancer researches have substantially confirmed this initial immunological BCG anti-tumor approach, adopted later until now as golden standard therapy for bladder cancer and have clarified the association between lymphocytes tumor infiltration (as determined by intratumoral BCG) and life expectation.

BCG use in human ovarian tumors may perhaps have represented a right research premise for other successive studies of immunotherapy.

Nevertheless the association of BCG with immunity anti-check point treatments might encourage further studies in patients resistant to anti PD1 and CTL-4 therapy given alone or showing autoimmune diseases in the course of such a therapy (60). BCG vaccine or its derivative may be still considered as useful tools in cancer immunotherapy.

Combination treatment of BCG with immune therapy anti CTLA-4 and anti PD-1 checkpoint blockade, which have distinct cellular mechanisms of action (42 , 43), may give a more active and potent anti-tumor immune response and may be tried by further studies also in ovarian cancer (42 , 43) where it is not understood and difficult to overcome the resistance of cancer to PD-1 / PD-L1 blockade (57). Recent findings in the experimental animal model look promising, highlighting the adaptive dynamic regulation of PD-L1 in response to BCG immuno-

therapy therapies and suggesting that a combination of BCG immunotherapy with PD-L1 blockade may be an effective antitumor strategy for improving treatment outcomes of BCa (58-61).

The use of *i.d.* or intratumor BCG especially SDW-BCG can still be considered as part of the tools to cure or, joined to other immunotherapeutic strategies to treat human tumors. However, a lot of experience still needs to be done in this direction and since the first results obtained in humans in the early seventies (62), after almost 50 years much remains to be understood and improved.

CONFLICT OF INTERESTS

The author declares that he has no conflict of interests.

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Are HLA-G polymorphisms associated to recurrent pregnancy loss?

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ABSTRACT

HLA-G molecule is considered to be involved in the regulation of the maternal-foetal interface immune tolerance, thus contributing to a healthy placentation. Some polymorphisms of the gene encoding for HLA-G are suggested to affect transcription and correlate with low levels of the soluble and membrane bound HLA-G protein. Patients affected with these polymorphisms are low secreting and may have pregnancy complications. We investigated the prevalence of 3'UTR 14-base pair insertion (I)/deletion (D) and of the single nucleotide polymorphisms (SNPs) 5'URR-725 C/G in recurrent miscarriage and healthy controls. A total of 286 couples affected with recurrent pregnancy loss (RPL) and 17 healthy couples were investigated for both polymorphisms. Couples underwent an accurate diagnostic flow chart in order to exclude causal factor of RPL and 137 couples were diagnosed as unexplained (URPL). The prevalence of the 3'UTR 14bp I/D polymorphism heterozygous was similar between URPL and control group. 3'UTR 14 bp D/D homozygotes were statistically higher in control group. Surprisingly, the prevalence of 3'UTR 14bp I/I homozygotes was similar between groups. Regarding 5'URR-725 C/G polymorphism in URPL the most prevalent haplotype was healthy homozygote C/C in combination with C/G. No association was found between the presence of homozygotes for the low secreting 5'URR-725 G/G polymorphism and URPL. Our results indicate that the evaluation of HLA-G polymorphisms is not adequately supported in clinical practice.

SOMMARIO

La molecola HLA-G è coinvolta nella regolazione della tolleranza immunitaria dell'interfaccia materno-fetale, contribuendo così a una sana placentazione. Si ipotizza che alcuni polimorfismi del gene che codifica per HLA-G influenzino la trascrizione e siano correlati a bassi livelli della proteina HLA-G solubile legata alla membrana. I pazienti affetti da questi polimorfismi sono a bassa secrezione e possono avere complicazioni durante la gravidanza. Abbiamo studiato la prevalenza dell'inserzione/delezione di 3'UTR a 14 paia di basi e dei polimorfismi a singolo nucleotide (SNP) 5'URR-725 C/G in aborti spontanei ricorrenti e controlli sani. Un totale di 286 coppie affette da interruzione di gravidanza ricorrente (RPL) e 17 coppie sane sono state studiate per entrambi i polimorfismi. Le coppie sono state sottoposte a un accurato diagramma di flusso diagnostico per escludere il fattore causale di RPL e 137 coppie sono state diagnosticate come inspiegabili (URPL). La prevalenza del polimorfismo I/D 3'UTR 14 paia di basi eterozigote era simile tra URPL e gruppo di controllo. 3'UTR 14 paia di basi D/D omozigoti erano statisticamente più alti nel gruppo di controllo. Sorprendentemente, la prevalenza di omozigoti 3'UTR 14 paia di basi I/I era simile tra i gruppi. Per quanto riguarda il polimorfismo 5'URR-725 C/G in URPL, l'aplotipo più diffuso era C/C omozigote sano in combinazione con C/G. Non è stata trovata alcuna associazione tra la presenza di omozigoti per il polimorfismo 5'URR-725 G/G a bassa secrezione e URPL. I nostri risultati indicano che la valutazione dei polimorfismi HLA-G non è adeguatamente supportata nella pratica clinica.

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Key words:

Recurrent miscarriage; HLAG; polymorphism; 3'UTR 14 bp I/D; 5'URR SNPs.

INTRODUCTION

Sporadic miscarriage is the most frequent complication of early pregnancy. Almost 70% of product of conception fail prior to life and most losses occur during the pre-implantation period (1). Recurrent pregnancy loss (RPL), defined as two or more spontaneous abortions prior to the 20th week of pregnancy (ESHRE 2017), occurs in about 2%-5% of otherwise healthy women (2). The aetiology of this condition is often multifactorial. After ruling out traditional causes, including chromosomal anomalies, anatomical abnormalities, endocrine disturbances and clotting disorders, in about 50% of RPL cases the pathophysiology of RPL remains unexplained. Therefore, these cases may be considered as unexplained recurrent pregnancy losses (URPL) (3).

Different immunological mechanisms take place at the foetal-maternal interface ensuring the protection of the semiallogenic fetus and the maintenance of pregnancy, thus, abnormal immunologic interactions may be responsible for some cases of otherwise unexplained RPL. Decidual T lymphocytes, decidual natural killer (dNK) cells, macrophages and antigens on the extravillous trophoblast, which is the external layer of trophoblast in contact with maternal tissue, may be involved in these interactions. In particular, during early placentation, the extravillous trophoblasts, migrating into the decidua, display a unique pattern of HLA class 1b molecules, with HLA-E, F and G predominating. The role of HLA-E and F are still under debate. HLA-G protein is highly expressed on extravillous trophoblast and may play a role on immune modulation at that level (4).

This protein is encoded by the nonclassical HLA-G locus. There are two forms of this protein: membrane-bound and soluble (sHLA-G). The membrane bound isoforms are four: HLA-G1, HLA-G2, HLA-G3, HLA-G4 and the soluble isoforms are three: HLA-G5 (or sHLA-G1), HLA-G6 (or sHLA-G2), HLA-G7 (or sHLA-G3). Both, membrane bound and soluble HLAG, exhibit immu-

nologic functions. HLA-G activates different pathways in uterine NK cells, macrophages and T cells and alters their immune function by interacting with leukocyte inhibitory receptors ILT2 (immunoglobulin like transcript) and KIRs receptors (killer immunoglobulin-like receptors) on dNK (decidual natural killer cells) inducing the secretion of Th2 cytokines and VEGF (5), with macrophages through ILT2 and ILT4 receptors, favouring the shift toward the immunomodulatory phenotype M2 macrophages and with the ILT2 receptor on CD8+ cells, inhibiting cytotoxicity (6). HLA-G binding to ILT4 on antigen-presenting cells, suppresses T-cell proliferation, via apoptosis (7). Moreover, HLA-G is not involved in T-cell suppression exclusively with its membrane-bound form, but also with a soluble isoform in maternal serum (8-10).

Polymorphism on the gene encoding for HLA-G antigen are associated with pregnancy complication such as recurrent pregnancy loss, recurrent implantation failure, and preeclampsia (11-13), but these reports are controversial, with other studies reporting a not significant correlation between HLAG polymorphism and RPL (14-16). Among its several polymorphisms in the non-coding region of the HLA-G gene, the 14bp ins/del polymorphism at 3' untranslated region (3'UTR) of HLA-G gene and the single nucleotide substitution (SNP) polymorphisms HLA5'URR-725 C/G play an important role in post-transcriptional regulation of HLA-G molecule. Individuals carrying those polymorphisms have dramatically lower sHLA-G protein level in plasma and are considered low secreting haplotypes (17). Several studies on the association between HLA-G 14 bp ins/del polymorphism and URPL have been performed. Low secretion of soluble HLA-G (s-HLA-G) from preimplantation embryos may be predictive of low implantation rate in *in vitro* fertilization, and low plasma levels of s-HLA-G may be associated with low implantation rate after *in vitro* fertilization and high risk of recurrent miscarriage (18, 19).

The role of the single nucleotide substitution polymorphism (SNPs) of the HLAG gene, substitution

of a C with G in 725 position (C/G), has been less studied in association with RPL. This polymorphism changes the amino acid sequence of the HLA-G protein and is also responsible for the low secreting haplotypes. In a study of SNPs in the 5' URR-725 C/G of the HLA-G gene in a cohort of Hutterite couples, Ober *et al.* observed an increased risk of abortion in couples where both members carried the 725G allele (OR 2.8) (11).

However, no clear consensus has been reached. Because of the inconsistent results from relatively small studies with underpowered to detect the effect of these polymorphisms. Recently two meta-analysis, including all relevant studies, have been conducted in order to define the influence of these polymorphisms in RPL, Wang *et al.* reported an association between the HLA-G14 bp insertion haplotype with RPL (OR 1.47 (1.13-1.91)) and an overall risk of RPL independent from the previous miscarriage number (17). In the other meta-analysis study (20) reported the same association but only in patients with three or more RPL.

Aim of this study is to investigate the association of low secreting polymorphism 3'UTR 14 bp I/D and 5'URR-725 C/G, in couples affected with RPL compared to healthy controls. To our knowledge this is the first study that investigates both polymorphisms in both partners.

MATERIALS AND METHODS

Study subjects

In this longitudinal case-control study, subjects were recruited for investigation and treatment at the outpatient clinic for diagnosis and treatment of RPL at the University of Tor Vergata, Rome, Italy from March 2014 to March 2018. Control couples were volunteers that accepted to enter the study and signed an informed consent. All couples recruited in the study were asked for permission to get blood or oral swap samples for DNA preparation and signed an informed consent.

The study sample included 286 couples with diagnosis of RPL according to ESHRE criteria (ESHRE 2017). All women underwent diagnostic flow chart for RPL and clinical features of control group are reported in **table I**:

- BMI;
- assessment of primary or secondary RPL;
- number of abortions;

Table I. Clinical features of RPL population.

| RPL population | |
|-----------------------|--------|
| Mean Age | 37 |
| Mean BMI | 23 |
| Mean Nr. of abortions | 3 |
| Mean Week of abortion | 7 |
| Primary RPL | 75.70% |
| Secondary RPL | 24.30% |

- average Week of abortion of RPL;
- karyotype of issue of conception (when available);
- karyotype of both partners;
- TSH, fT3, fT4;
- Anti-thyroglobulin antibodies, Anti-thyroid peroxidase antibodies;
- prothrombin 20210 mutation (factor II mutation);
- factor V Leiden mutation;
- protein S and protein C, ATIII;
- Lupus Anticoagulant antibodies (according to Bradford Hill recommendations);
- anticardiolipin antibodies (according to Bradford Hill recommendations);
- anti-beta-2-glycoproteins antibodies (according to Bradford Hill recommendations);
- ENA, ANA, DsDNA, antitransglutaminase ab;
- genotyping of HLA-G for haplotypes.

After the full evaluation of the 286 couples, according to the diagnostic flow chart, 149 couples were multifactorial RPL and 137 couples resulted as unexplained (URPL). Clinical features of RPL patients are summarized in **table I**.

Control population

A total of 17 couples were recruited to this study. The inclusion criteria were:

- no abortions;
- 2 or more live births;
- no obstetrical complications;
- age < 65 years.

Clinical features controls are reported in **table II**.

DNA isolation

DNA was extracted from 200 ml of peripheral blood or oral swap in EDTA according to the Qiagen DNA mini kit (Sambrook *et al.*, 2000). PCR amplification of each region of interest was performed using the outer oligonucleotide primers. Mutation analysis was carried out by direct sequencing of PCR products

Table II. Clinical features of control population.

| Control population | |
|-------------------------|------|
| Age | 48 |
| Mean BMI | 25 |
| Mean nr. of abortion | 0 |
| Mean nr. of pregnancies | 2 |
| Obstetric complication | None |

using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA), according to the protocol provided by the manufacturer.

Amplification and sequencing of HLAG-gene

For sequence analysis, 20 ng of purified PCR products were sequenced by direct cycle sequencing using fluorescent-labelled dideoxy terminators (Big Dye Terminator Cycle Sequencing Ready Reaction Kit; Applied Biosystems), according to the manufacturer's protocol. The reaction conditions were as follows: 25 PCR cycles, a denaturation step of 10 s at 96 °C, annealing for 5 s at 50 °C and extension for 4 min at 60 °C. Sequencing products were then purified using Centre-Sep® columns (Princeton Separation) for unincorporated dye terminator removal, according to the protocol provided by the manufacturer. Five microliters of each Centri-Sep® purified product were then added to 15 ml of Hi-Di Formamide (Applied Biosystems), heat-denatured at 90 °C for 4 min and run on ABI Prism® 310 automated DNA sequencer (Applied Biosystems). The sequences obtained were then compared with the wild type controls using Sequence Navigator® Software (Applied Biosystems) for mutation analysis. The minisequencing reaction was performed, starting from 10 ng of the same purified PCR product subjected to sequence analysis, using ABI Prism® SnaPshot Multiplex Kit (Applied Biosystems). The reaction volume was 10 ml, including 5 ml of Ready Reaction Premix and 10 pmol of each minisequencing primer. The reaction conditions were as follows: 25 PCR cycles, denaturation step of 10 s at 96 °C, annealing for 10 s at 50 °C and extension for 30 s at 60 °C.

In the minisequencing technique, a primer extension reaction is performed, starting from a specific primer that is designed to anneal directly adjacent to the mutation site, by the incorporation of a single fluorescent dideoxynucleotide (ddNTP), which is complementary to the variant base in the template. Since the primer is designed to anneal directly adjacent to the variant base of interest, and the reaction mix

does not include dNTP, incorporation occurs only at a single site. This process is repeated in successive rounds of extension and termination to generate the fluorescently labelled fragment for analysis. After the primer extension reaction, 1 ml of minisequencing product was mixed with 15 ml of Hi-Di Formamide and denatured for 4 min at 90 °C. The samples were then resolved and detected by 15 min (per blastomere) of capillary electrophoresis on automatic DNA sequencer ABI Prism® 310, using POP-4® polymer and 47 cm x 50 mm capillaries. Thus, the mutation sites could reliably be differentiated among homozygotes wild types, homozygotes mutants or heterozygotes, by the dye-labelled ddNTP incorporated. To reveal the electrophoresis data, the peak signal was analysed with GeneScan® Analysis Software (Applied Biosystems); the dye colour of the fragment was used to identify the nucleotide of interest. For the minisequencing technique, colour was assigned to individual ddNTP as follows: green/A, black/C, blue/G, red/T. For sequencing, instead colours were assigned as follows: green/A, black/G, blue/C, red/T. The minisequencing reaction produces one (homozygote) or two (heterozygote) peaks depending on the genotype at this locus.

PCR amplification and minisequencing primers design

The PCR strategy consisted of initial multiplex external amplification followed by nested PCR, specific for each region involving mutations. After cell lysis and neutralization, 1.5 mmol/l MgCl₂, 200mmol/l of each dNTP (Roche Diagnostic, Italy), 2.5 IU AmpliTaqPolymerase (Applied Biosystems), 10 pmol of each outer primer, were added to each tube, for a total volume of 50 ml. The first round of PCR involved a 96 °C denaturation temperature in the first 10 cycles as a means to reduce ADO (21), followed by a subsequent denaturation temperature of 94 °C in 25 remaining cycles. Each round of PCR was preceded by an initial 4 min denaturation step at 94 °C and followed by a final extension step of 10 min at 72 °C. The extension temperature depended on the specific primers used. PCR positive heterozygous controls containing a single lymphocyte (two control cells for each genotype) isolated from carriers were also added.

For the second round of DNA amplification, 2 ml of the primary PCR reaction product were added to another tube containing 5 ml of 10QPCR Buffer II (500 mmol/l KCl, 100 mmol/l Tris HCl, pH 8.3; Applied Biosystems), 1.5 mmol/l MgCl₂, 200 mmol/l of each

dNTP (Roche Diagnostic), 2.5 IU AmpliTaqPolymerase (Applied Biosystems), 10 pmol of each inner primer, in a total volume of 50 ml, and the tubes were cycled as above on a GeneAmpa PCR System 9700 (Applied Biosystems).

To monitor successful amplification, 5 ml of each PCR product was subjected to electrophoresis for 5 min at 150 V on 2% agarose gel in 1QTris ± borate/ EDTA buffer stained with 0.5 mg/ml ethidium bromide.

To avoid participation in the subsequent primer-extension reaction, primers and unincorporated dNTP were removed from PCR products by performing Microcon 100 (Amicon, USA) purification, according to the manufacturer's protocol.

Mutation analysis was carried out only on positively amplified blastomeres, using two different techniques simultaneously: sequence analysis and minisequencing.

After mutation detection, minisequencing primers were designed for each mutation investigated with the aid of Primer Express® software (Applied Biosystems) and initially tested on sequenced PCR products. Desalted primers were purchased from MWG-Biotech (Germany). The guidelines for primer design included the following parameters:

1. Design primers 18 nucleotides in length or greater with melting temperatures of b 45 °C;
2. check primers for possible extendable hairpin structures and for extendable dimer formation between primers;
3. high performance liquid chromatography purification of primers is recommended for oligonucleotides longer than 30 nucleotides;
4. use primers that are complementary to the negative (±) DNA strand, if the positive (+) DNA strand is difficult to assay;

5. in multiplex reaction, primers must differ significantly in length so that overlap between the final products will be avoided.

A difference of 4 ± 6 nucleotides between primer lengths is recommended. The length of a primer can be modified by the addition of non-homologous polynucleotides at the 5' end. Poly (dT), poly (dA), poly (dC) and poly (dGACT) are 5' non-homologous tails, which are predicted to have minimal secondary structures. This procedure is summarized in figure 1.

Statistical analysis

Full data were entered in an Excel sheet (Microsoft, Redmond, Washington - USA) and they were analysed by a statistics pack for social sciences Windows, version 1.5 (SPSS, Chicago, Illinois, USA).

The interaction between the presence/absence of a variables at any specific group membership was tested using a Fisher's exact test. OddsRatio (OR), a measure of association between the two variables and confidence interval (CI), asymptotic confidence interval for the odds ratio were reported. Statistically meaningful are the values with p < 0.05.

All charts were designed by Excel (Microsoft, Redmond, Washington, USA).

RESULTS

Patients affected with RPL underwent a very extensive diagnostic flow chart and more investigations were performed beyond the traditional factors of RM (genetic, anatomic and aPL syndrome) since they were included in a research program. The prevalence of different aetiologies is summarized in figure 2.

In particular 21 out of 286 (7%) were affected with aPL syndrome, 81 out of 286 (28%) had thyroid abnormalities, 5 out of 286 (2%) had uterine Mullerian anomalies, 2 out of 286 (1%) had chromosomal abnormalities, 16 out of 286 (6%) had hereditary thrombophilia, 24 out of 286 (8%) had associated causes of RM. No causal factor of RPL was found in 137 and they were classified as idiopathic or unexplained URPL.

The statistical analysis in the prevalence of HLAG polymorphisms was performed in 137 unexplained recurrent pregnancy loss couples (URPL), compared to 17 healthy controls.

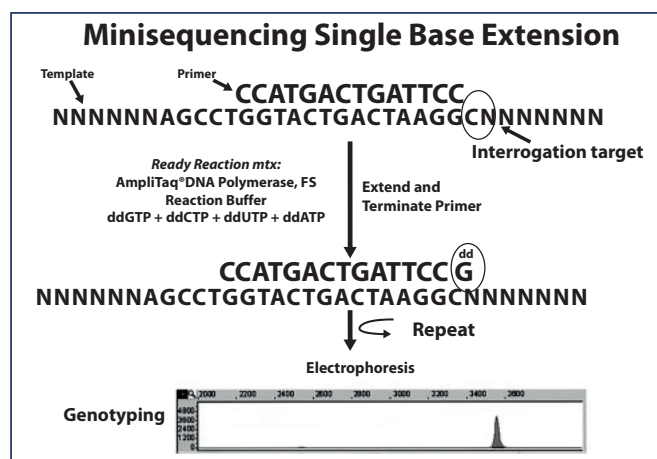


Figure 1. Dna extraction and amplification.

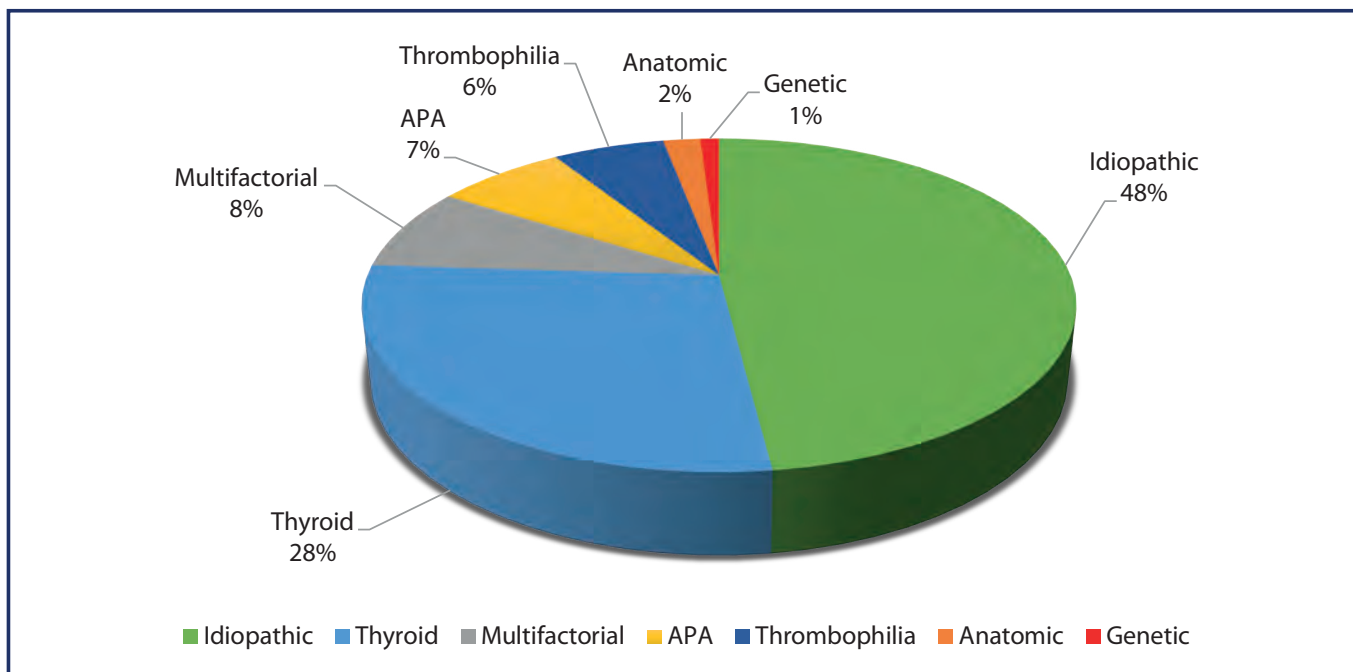


Figure 2. Aetiologies of RPL in 286 selected patients after diagnostic flow chart.

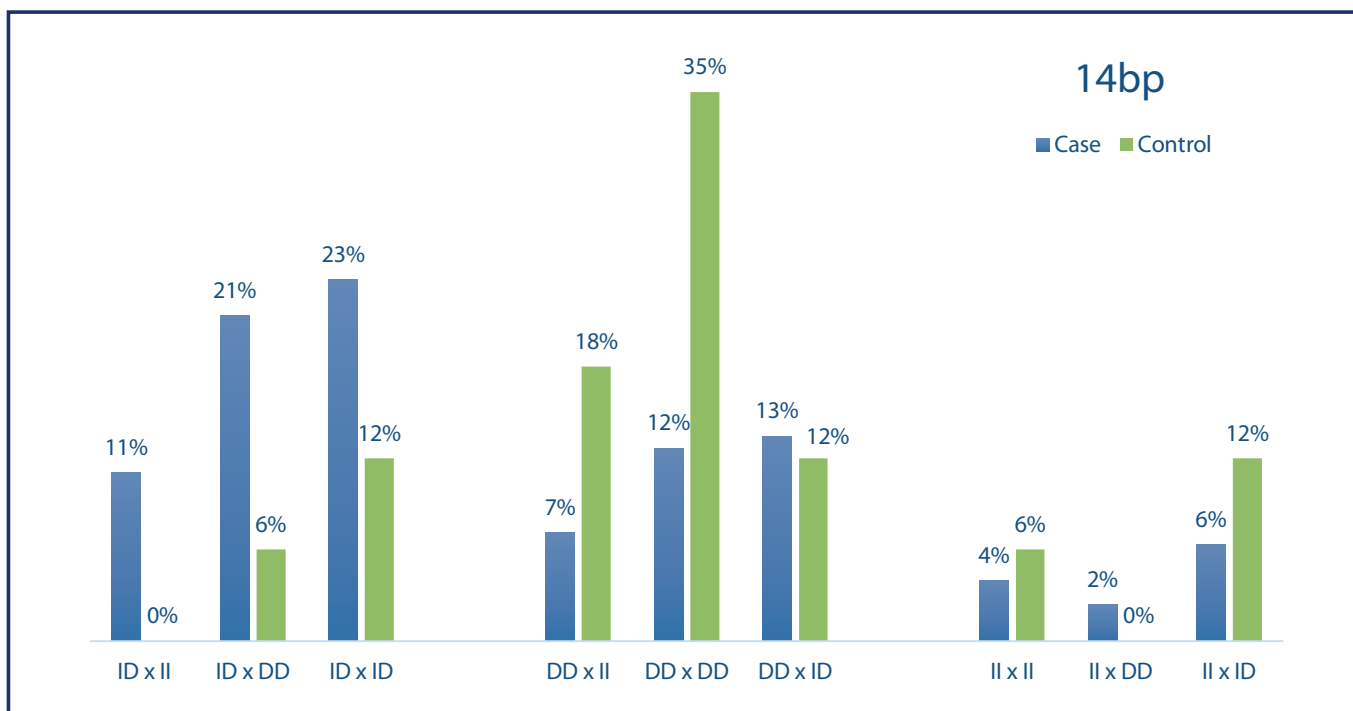


Figure 3. Comparison of 14bp I/D haplotypes between URPL couples vs control group.

3'UTR 14 bp

In URPL population, the low secreting haplotype of 3' UTR 14bp (I/D) has the highest incidence (101 partners out of 274 total subjects in case group, 37%). In control population, the normal secreting haplotype of 3'UTR 14bp (D/D) was the most prevalent

haplotype (18 partners out of 34 total subjects in control group, 53%) (table III).

In URPL population, most of the couples have at least one low secreting haplotype: 31 couples (23%) have the association I/D x I/D and 28 couples (20%) have the association I/D x D/D. Nonetheless, the prevalence of 14bp heterozygous combi-

nations in URPL, compared to healthy group, did not reach statistical significance.

In control population, 6 couples out of 17 (35%) were homozygous for the normal secreting association D/D x D/D, compare to 12% (16 out of 137) in URPL couples. We found a statistically significant higher prevalence of homozygous combination D/D x D/D in couples with normal pregnancy outcome compare to URPL (p = 0.018; OR = 0.242) (table III, figure 3).

5' UTR 725

Normal secreting haplotypes combination CC x CC was the most prevalent both in control (29%) and URPL (53%) but do not reach statistical significance. A significantly higher prevalence of the C/C x C/G combination was found in healthy controls (29%) compare

Table III. Prevalence of 14 bp I/D haplotypes in URPL couples vs controls.

| Variable (14 bp) | Patients, n (%) | Controls, n (%) | p - value | OR [CI] |
|------------------|-----------------|-----------------|-----------|--------------------------|
| ID x II | 15 (11%) | 0 (0%) | Ns | - |
| ID x DD | 28 (20%) | 1 (6%) | Ns | - |
| ID x ID | 31 (23%) | 2 (12%) | Ns | - |
| DD x II | 10 (7%) | 3 (18%) | Ns | - |
| DD x DD | 16 (12%) | 6 (35%) | 0.018* | 0.242 [0.079 - 0.745] |
| DD x ID | 18 (13%) | 2 (12%) | Ns | - |
| II x II | 6 (4%) | 1 (6%) | Ns | - |
| II x DD | 4 (3%) | 0 (0%) | Ns | - |
| II x ID | 9 (7%) | 2 (12%) | Ns | - |

to URPL (10%) (p = 0.039; OR = 0.273). Surprisingly, we do not find any difference in the prevalence of the low secreting combination GG x GG between URPL (1%) and controls (6%) (Ns) (table IV, figure 4).

CONCLUSIONS AND DISCUSSION

For more than two decade the HLAG molecule has been considered as an immune regulator towards tolerance of the semi-allogenic embryo (4, 14, 22, 23). An elevated HLA-G expression is also involved in the mechanisms to evade immune surveillance in viral infections (24), tumours immune escape and as a marker in transplantation outcome (25). Moreover, HLA-G has been detected in seminal plasma, and paternal s-HLAG seems to play a role in the implantation process of the blastocyst through the inhibition of CD4 T cells proliferation and the induction of T Regs

at the endometrial level (26). An association between low levels of s-HLAG in maternal plasma and the development of preeclampsia has also been reported (9, 27). As above mentioned both polymorphisms of the 3' UTR untranslated region 14 bp and single nucleotide substitution of the 5' URR- 725 C/G affect the expression and function of s-HLAG, and correlate with pregnancy complications such as preeclampsia (27) and recurrent pregnancy loss (14, 22).

In the present study the prevalence of the HLAG gene polymorphism was evaluated in couples with URPL and in controls. We analysed the presence or absence of the 14bp segment at the 3' UTR region, the single nucleotide substitution C/G at the 5' URR-725 segment of the gene, and the haplotype distributions among couples with URPL and controls. To our knowledge, this is the first study that evaluates both polymorphisms in both partners, and not only in the female partner.

Our data fail to demonstrate a clear association between URPL and HLAG gene polymorphisms. Analysing the 14bp polymorphism of HLAG at 3' UTR region, no differences were found in the prevalence of the low secreting haplotypes between URPL and normal control couples. Interestingly, the prevalence of the normal secreting haplotypes (D/D x D/D) was higher in healthy couples suggesting a possible positive effect on trophoblast invasion (figure 3). Based on this observation, it could be speculated that the presence of low secreting haplotypes does not influence implantation process whereas the normal secreting haplotypes could have a protective role, improving pregnancy outcomes. Conversely, the evaluation of the 5' URR - 725 SNP polymorphisms in our series provided conflicting results. No significant differences were found between URPL patients and normal control couples. In particular, the prevalence of the low secreting haplotypes was similar

Table IV. Prevalence of - 725 C/G haplotypes in URPL couples vs controls.

| Variable (14 bp) | Patients, n (%) | Controls, n (%) | p - value | OR [CI] |
|------------------|-----------------|-----------------|-----------|--------------------------|
| CC x CC | 73 (53%) | 5 (29%) | ns | |
| CC x GG | 5 (4%) | 1 (6%) | ns | |
| CC x CG | 14 (10%) | 5 (29%) | 0.039* | 0.273 [0.084 - 0.890] |
| GG x CC | 4 (3%) | 0 (0%) | ns | |
| GG x GG | 0 (0%) | 1 (6%) | ns | |
| GG x GC | 2 (1%) | 1 (6%) | ns | |
| CG x CC | 22 (16%) | 3 (18%) | ns | |
| CG x GG | 4 (3%) | 0 (0%) | ns | |
| CG x CG | 13 (9%) | 1 (6%) | ns | |

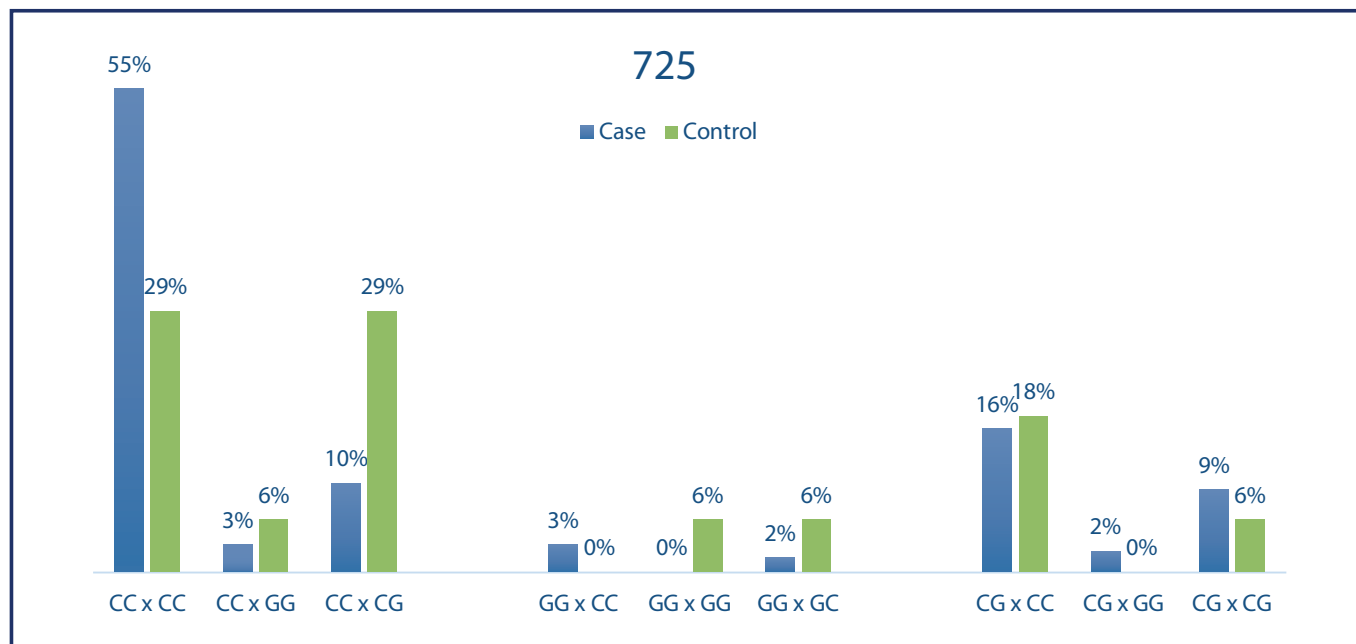


Figure 4. Comparison of -725 C/G haplotypes between URPL couples vs control group.

in both groups and, surprisingly, in our population the normal secretive haplotype (C/C) was the most prevalent. Moreover, we found a slightly increased prevalence of the CC x CG polymorphism in control group. These data, although in contrast with previously reported evidence, seem to suggest that the 5' URR - 725 SNP polymorphisms should not be considered among causal factors of RPL. This discrepancy with the previous papers, may be due to the fact in most studies that 5' URR 725 SNP polymorphism evaluation was performed only in the female partner. Nonetheless, the paper by Coulam and Roussev (29) reported a higher incidence of G/G haplotype in RPL couples. These conflicting data suggest that further well designed studies including a higher number of cases and controls, are needed to ascertain the role of 5'URR 725 polymorphism in RPL.

Another confounding factor in the role of HLAG polymorphisms may be due to the multifactorial aetiology of RPL. Some studies did not exclude other aetiologies of their population and this may represent a bias (30). For this reason, our patients underwent a very extensive diagnostic flow chart in order to exclude traditional factors of RPL. Patients included in our studies had other associated aetiologies in 52% and were URPL in 48% of cases. The statistical comparison of HLAG polymorphisms prevalence was, therefore, performed between 137 URPL couples and controls. An important drawback of our study is due to the small number of patients of control group. Even though the prevalence

of HLAG haplotypes found in our control patients was similar to the one reported in other papers with more control patients (19).

In conclusion, this is the first report to perform a comprehensive analysis of 3' URT 14 bp I/D polymorphisms and 5' URR 725 C/G SNP in both partners in a population on URPL. Our study does not find any association neither with heterozygous 14 bp polymorphism and URPL, nor with homozygous that is supposed have impaired levels of sHLAG. The association between 5' URR 725G heterozygous polymorphism and URPL, provides conflicting results, since the low secreting association CC x CG was more prevalent in control group. The role of HLAG in the pathogenesis of RPL is challenged. In basic research, deeper analysis is necessary on the role of immune cells, cytokines and receptor interaction and molecules at the maternal-foetal interface. In clinical studies, well-designed and powered trials are needed to examine HLA alleles, polymorphism and sHLAG protein level in couples experiencing RPL. Actually, according to the ESHRE guidelines and to our data, the evaluation of HLAG polymorphisms is not adequately supported in clinical practice.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Grade of satisfaction and tolerability of a medical device based on *P. acnes* in adults waiting for destructive treatments for genital warts

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ABSTRACT

Vulvar and vaginal warts (genital warts – GW), also known as condylomata acuminata, are one of the clinical manifestations of human papillomavirus (HPV) infection. Oxidative stress may have an important role in the pathogenesis of GW. A preparate of bacterial lysate based on *Propionibacterium acnes* (*P. acnes*) showed a potent antioxidant activity *in vitro*, suggesting a potential role in the treatment of GW. The aim of the present study was to evaluate the grade of satisfaction of patients who were waiting for destructive treatment, prospective enrolled and treated for 60 days with a *P. acnes* lysate. The effect on the number, location of the lesions and the quadrants involved was also evaluated waiting destructive approach. Anamnestic data were collected and a vulvoscopy was performed with an accurate description of the lesions at the first visit and after 30-60-90 and 180 days of treatment. During the study period, 69 women, fulfilling the study inclusion/exclusion criteria, constituted the study cohort. *P. acnes* preparation showed a high tolerability and a high grade of satisfaction. Moreover, all patients included decided to wait at least 60 days before being subjected to any destructive treatment, which was then necessary only in 31.8% of cases. The results

SOMMARIO

La condilomatosi vulvare e vaginale (CV) è una delle manifestazioni cliniche dell'infezione da papillomavirus umano (HPV). Lo stress ossidativo può avere un ruolo importante nella patogenesi della CV. Un preparato di lisato batterico a base di *Propionibacterium acnes* (*P. acnes*) ha mostrato una potente attività antiossidante *in vitro*, suggerendo un potenziale ruolo nel trattamento della CV. Obiettivo dello studio è stato quello di valutare il grado di soddisfazione e la tollerabilità dopo somministrazione di un preparato contenente lisato di *P. acnes* in pazienti in attesa di trattamento distruttivo per condilomatosi. Le pazienti arruolate sono state trattate per 60 giorni consecutivi ed è stato valutato l'effetto sul numero, sulla sede delle lesioni e sui quadranti coinvolti, l'insorgenza di effetti collaterali, il grado di soddisfazione e la tollerabilità. Alla prima visita sono stati raccolti dati anamnestici ed è stata eseguita una vulvosopia con descrizione accurata delle lesioni. Le pazienti sono state rivalutate a 30, 60, 90 e 180 giorni dall'inizio del trattamento. La coorte di studio era costituita da 69 donne, che osservavano i criteri di inclusione/esclusione dello studio. La preparazione di *P. acnes* ha mostrato un'alta tollerabilità e un alto grado di soddisfazione. Tutte le pazienti incluse

obtained with this preliminary study enable to indicate this therapy as possible innovative option in the treatment of GW. A prospective blinded RCT will be necessary to evaluate the *P. Acnes* preparation efficacy in the treatment of condylomatosis.

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hanno deciso di attendere almeno 60 giorni prima di essere sottoposte a qualsiasi trattamento distruttivo, che si è rivelato necessario solo nel 31,8% dei casi. Al termine del trattamento, è stata osservata una risposta completa, parziale o assente alla terapia rispettivamente in 37 (61.6%), 15 (25%) e 8 pazienti (13.4%). I risultati ottenuti con questo studio preliminare consentono di indicare questa terapia come possibile opzione innovativa nel trattamento della CV. Sarà necessario un RCT in cieco per valutare la potenziale efficacia della preparazione di *P. acnes* nel trattamento della condilomatosi.

Key words:

Genital warts; Propionibacterium acnes; condylomata acuminata; oxidative stress; therapy.

INTRODUCTION

Vulvar and vaginal warts (genital warts – GW), also known as condylomata acuminata, are one of the clinical manifestations of human papillomavirus (HPV) infection. According to their oncogenic potential, low-risk HPV types 6 and 11 are responsible for over 90 percent of the GW (1), which are one of the most common sexually transmitted diseases (2-4) worldwide.

Although the vaccination with quadrivalent and nonavalent vaccines reduce the incidence of GW in women and men (5), the vaccination coverage in Italy was in 2017 significantly below the optimal threshold set by the National Vaccinal Prevention Plan, with 56% estimated vaccine coverage among females aged 12 (6). In a large European survey conducted on general adult populations (18-45 years), the self-reported lifetime history of GWs was 10% (7).

Following initial clinical manifestation, approximately 30 percent of all warts will spontaneously regress within the first four months of infection (8). Unfortunately, long-term remission rates remain largely unknown, and most GW will recur within three months of infection, even after undergoing appropriate treatments (9). Significant risk factors for long-term persistence include host immunosuppression, infection with high-risk HPV subtypes, and an older patient age (9, 10). A prolonged infection with HPV leads to chronic inflammation which can locally cause oxidative stress (11). Oxidative stress occurs as a result of unbalance between the

oxidant and the antioxidant system (12) and constitutes the basis of many inflammatory skin disease and skin cancer (13, 14). Oxidative stress may also have an important role in the pathogenesis of genital warts, as previously described by Cockuk *et al.* (12). Briefly, the authors found that the activities of glutathione peroxidase and catalase and the level of malondialdehyde (MDA, a well-known indicator of oxidative stress), were significantly higher in patients with genital warts than in healthy subjects. The current options available for the treatment of GW are meant to eliminate or to reduce the warty growth, rather than to determine the clearance of the underlying viral infection (8). Wide ranges of therapies are currently used, with variability with respect to cost, side-effect profiles, dosing schedules, duration of treatment, and overall effectiveness (8). Several studies reported the successful use of topical or systemic zinc-based antioxidants for the treatment of non-genital warts (15, 16) and the efficacy of catechins-based formulations on the external genital and perianal warts via inhibitory effects of enzymes related to oxidative stress (17). Furthermore, a prepare of bacterial lysate based on *Propionibacterium acnes* (*P. acnes*), a gram positive, pleomorphic, strictly anaerobic bacteria, showed a potent antioxidant activity *in vitro*: in particular, such prepare counteract the increase of MDA and nitrites in hydrogen peroxide-stimulated murine fibroblasts culture (Cuzzocrea, Messina, Italy, data not published), suggesting a potential role in the treatment of GW.

Alternatively, GW can be removed by scalpel or scissor excision or they can be destroyed by electrosurgery or carbon dioxide laser ablation. However, not all these treatments have showed completely satisfactory results; a high recurrence rate after their utilization, as well as definitive scars and sequelae, are described with a recurrence rate of about 25% for destructive therapy too (8).

The aim of the present study was to evaluate the grade of satisfaction of patients treated with *P. acnes* lysate (Immunoderm®, Depofarma, Mogliano Veneto, TV, Italy), who were waiting for destructive treatment for GW; the effect on the number, location of the lesions and the quadrants involved was also evaluated waiting destructive approach.

METHODS

A prospective multicentric cohort study (January 2018-September 2019) was conducted on patients who were waiting for destructive treatments for vulvo-perineal condylomatosis, consecutively enrolled and treated for 60 days with a commercially available preparations based on *P. acnes* (Immunoderm®, Depofarma, Mogliano Veneto, TV, Italy). The *P. acnes* preparation used was a medical device with marketing authorization number AIC-972778029 of the Italian Medicines Agency (AIFA) and European Community certification mark approved for use in patients with symptoms related to GW or subjected to any treatment for GW.

At inclusion, all eligible patients signed an informed consent, showing the technical and scientific basis of the research project and granting their permission to data collection and to administration of *P. acnes* preparation. The study was conducted in accordance with the Declaration of Helsinki (1964). The information collected and the clinical observations from the clinical examination were in possession only of those involved with the project and the patients, if they want. All included patients were over 18 years old and had a first diagnosis of multiple genital warts (three or more), all with a diameter less than one centimeter. Patients with the presence of cervico-vaginal warts, HIV positivity, immunosuppression (transplant patients, steroid therapy or with autoimmune disease), pregnancy and the onset of lesions for more than three months from the potential recruitment were excluded. All the cases with diagnostic uncertainty, which did not allow for a clinical diagnosis (*e.g.*, pigmented,

indurated, affixed to underlying tissue, bleeding, or ulcerated lesions) or with suspicion of precancer or cancer were excluded.

Visit 1 (T_0)

At the first clinical evaluation, anamnestic data were collected (age, BMI, HIV positivity, smoking habit and number of cigarettes/day, number of previous partners, number of previous pregnancies, concomitant pathologies, ongoing therapies, period of onset of the lesions). A vulvoscopy was performed with an accurate description of the lesions and a cervico-vaginal examination was performed to exclude the presence of cervico-vaginal warts. Typical warts were identified according to the clinical features reported by the International Union Against Sexually Transmitted Infections (IUSTI) (18): superficial papular lesions of 1-5 mm diameter, flat or pedunculated, solitary or multiple (also forming larger plaques). GW were identified as soft, raised masses, with smooth, verrucous, or lobulated aspects that could appear as pearly, filiform, fungating, or plaque-like eruptions often with surface showing finger-like projections, generally nonpigmented.

In accordance with the recommendations of the IUSTI (18) and of the Centers for Disease Control and Prevention (CDC) (19), the diagnosis of anogenital warts was made by visual inspection in all the cases with typical anogenital warts. Biopsy was not necessary for typical anogenital warts as recommended by afore mentioned guidelines (18, 19). The location of warts and the number of lesions (near to clitoris, right labia majora, left labia majora, right labia minora, left labia minora, vestibule, perineum, anus and perianus) and the number of quadrants involved were recorded. At the end of the first evaluation, topical preparation based on *P. acnes* was prescribed, 2 applications per day for 60 days, to all included patients attending for destructive treatment for GW. During the 60 days period, unsatisfied patients were free to discontinue topical application and undergo immediately destructive treatment.

Visit 2 (T_1 : 30 days after recruitment)

A vulvoscopy was performed, reporting the number, location of the lesions and the quadrants involved, according to the modalities of the visit 1. The patient's satisfaction, also indicating resolution of specific symptoms, was assessed with a score from 1 to 5 corresponding to the following catego-

ries (insufficient; sufficient; discreet; good; excellent). Simultaneously, the response to the administration was categorized as complete (complete absence of warts), partial (persistence of at least one condyloma) or unchanged (number of warts unchanged from diagnosis). The possible occurrence of adverse events was recorded, also reporting the type, the period of onset and the need of any treatment. Local tolerability was assessed with a score from 0 to 5 corresponding to the following categories (no erythema; barely noticeable erythema; slight erythema without edema; moderate erythema and edema with or without papules; accentuated erythema or edema with or without papules; accentuated erythema or edema with blisters).

Visit 3 (T₂:60 days after recruitment), visit 4 (T₃:90 days after recruitment) and visit 5 (T₄:180 days after recruitment)

At visit 3, a vulvoscopy was performed with the same modalities of previous controls, and response to the *P. acnes* administration was also categorized. Patient's satisfaction and tolerability were recorded. Complete response patients were sent to follow-up, one month after the end of therapy (90 days from recruitment – visit 4/T₃) and four months from the end of administration (180 days from recruitment – visit 5/T₄), while patients with partial/unchanged response were subjected to destructive therapy, with subsequent follow-up 1 month (90 days from recruitment – visit 4/T₃) and 4 months from destructive treatment (180 days from recruitment – visit 5/T₄). The execution of destructive therapies in recurrence cases at T₃ or T₄ was recorded. The study protocol is shown in **table I**.

Outcome analysis

The degree of local tolerability, the patient's satisfaction and the possible occurrence of adverse events were analyzed. The rate of patients with complete, partial or unchanged response, the median number of GW and of quadrants involved after Immunoderm® topical administration at T₂, and the number of patients who needed destructive treatment were also recorded.

All the statistical analyses were performed using MedCalc for Windows version 12.7.0 (Medcalc®, MedCalc Software bvba, 2013, Ostend, Belgium). Continuous parametric variables were expressed as mean (\pm standard deviation); nonparametric variables were ex-

Table I. Study protocol.

| | | |
|---|--|--|
| Visit 1 (T₀) | 1. First clinical evaluation with collection of anamnestic data 2. Vulvoscopy 3. Prescription of <i>P. Acnes</i> preparation | |
| Visit 2 (T₁) 30 days | 1. Vulvoscopy 2. Evaluation of patient's satisfaction, local tolerability, response to therapy and occurrence of adverse events | |
| Visit 3 (T₂) 60 days | 1. Vulvoscopy 2. Evaluation of patient's satisfaction, local tolerability, response to therapy and occurrence of adverse events | |
| | Complete response to <i>P. Acnes</i> therapy | Partial/unchanged response to <i>P. Acnes</i> therapy |
| Visit 4 (T₃) 90 days | 1. Vulvoscopy | 1. Destructive therapy |
| Visit 5 (T₄) 180 days | 1. Vulvoscopy | 1. Destructive therapy |

pressed as median and range after testing for normal distribution. The significance of couples' comparisons between treatment sessions was analyzed using the Wilcoxon test for non-parametric data. A $p < 0.05$ was considered statistically significant.

RESULTS

During the study period, 69 women, fulfilling the study inclusion/exclusion criteria, constituted the study cohort. At T₀, the mean age was 35.0 ± 7.5 SD (range: 19.0-57.0) and the mean BMI was 23 ± 4.0 SD. Twenty-five patients (35.2%) were tobacco users with a median of 10 cigarettes (range 6-20) per day. The median number of sexual partners was 3 (range: 1-10); one patient suffered from epilepsy and one patient reported thyroid disorder, both taking medical therapy. Only the 28.9% of patients (20 patients) had almost one previous pregnancy (50% one pregnancy, 50% two pregnancies). The mean period of onset of the lesions before diagnosis was 40.6 days (± 34.6 SD). The incidence of at least one GW, according to sites, at the diagnosis (T₀) is reported in **table II**.

At T₀, the median number of lesions for each patient was 4 (range: 3-40) with a median number of quadrants involved of two areas (range: 1-5).

At T₁, data of two cases were not available. Median patient's satisfaction was 3 (discreet) (range: 1-5) and 51/67 (76.1%) of patients reported a discreet, good or excellent grade of satisfaction. A high local tolerability with no erythema or barely noticeable erythema was present in 85% of cases (57/67) with a median local tolerability of 0 (no erythema) (range: 0-2). No adverse side effects were recorded after 30 days of

Table II. Incidence of GW, according to sites at the diagnosis (T₀).

| Sites | Number of patients |
|--------------------|--------------------|
| Near to clitoris | 12 (17.4%) |
| Right labia majora | 21 (30.4%) |
| Left labia majora | 24 (34.8%) |
| Right labia minora | 15 (21.7%) |
| Left labia minora | 12 (17.4%) |
| Vestibule | 24 (34.8%) |
| Perineum | 30 (43.5%) |
| Anus and perianus | 28 (40.6%) |

therapies (T₁). The median number of lesions for each patient was 2 (range: 0-40) with a median number of quadrants involved of one area (range: 0-5). A complete, partial, or absent response to *P. acnes* administration was observed in 14 (20.9%), 37 (55.2%) and 16 (23.9%) patients, respectively. All patients, even with persistence of GW, decided to do not interrupt local therapy for another 30 days before undergoing a possible destructive treatment. No patients decided to be subjected to destructive therapy at T₁.

At T₂, at the end of 60 days of therapy, data about 60/69 patients (86.9%) are available; median patient's satisfaction was 4 (good) (range: 1-5) and 50/60 patients (83.3%) reported a discreet, good or excellent grade of satisfaction). A sufficient (5/60-8.3%) or insufficient grade (5/10-8.3%) of satisfaction was recorded only in the 8 patients with absent response to therapy and in 2 cases with partial response. No significant differences about patient's satisfaction was found after 30 or 60 days of therapy (p = 0.43). Local tolerability at T₁ was 0 (no erythema) (range: 0-2) with no erythema or barely noticeable erythema in 96.6% of cases (58/60). A significant improvement in local tolerability was found after 30 and 60 days of therapy (85% vs 96.6%, p = 0.05). No adverse effects were recorded after 60 days of therapies (T₂).

The median number of lesions for each patient was 0 (range: 0-9) with a median number of quadrants involved of one area (range: 0-5). A complete, partial, or absent response to therapy was observed in 37 (61.6%), 15 (25%) and 8 (13.4%) patients, respectively. A significant reduction of the median numbers of GW (4 vs 0 – IR: 3-5 vs 0-2; p < 0.001) and of the number of quadrants involved (2 vs 0 – IR: 1-3 vs 0-2; p < 0.001) were recorded (see figures 1, 2).

Among the 23 patients with partial or absent response after 60 days of therapy, 20 were subjected to destructive therapy (16 laser vaporization, 4 diathermocoagulation). Three patients decided to continue the follow-up.

Data about follow-up at 90 days after recruitment (T₃) are available in 43/69 cases (62.3%): no GW were observed in 40/43 cases (93%). Two cases with persistent GW at T₃ were the same cases with persistence at T₂ and were treated with destructive therapy (laser vaporization). One patient decided to continue follow-up. Data about follow-up at 180 days after recruitment (T₃) are available in 27/69 cases (39.1%) and showed 1 persistence of GW in one patient who refused therapy at the 90 days follow-up (T₃). No cases of relapse of GW were recorded at T₃ and T₄ follow-up.

DISCUSSION

Genital warts have quality of life (20-23) and significant socio-economic impact, as it is the most common

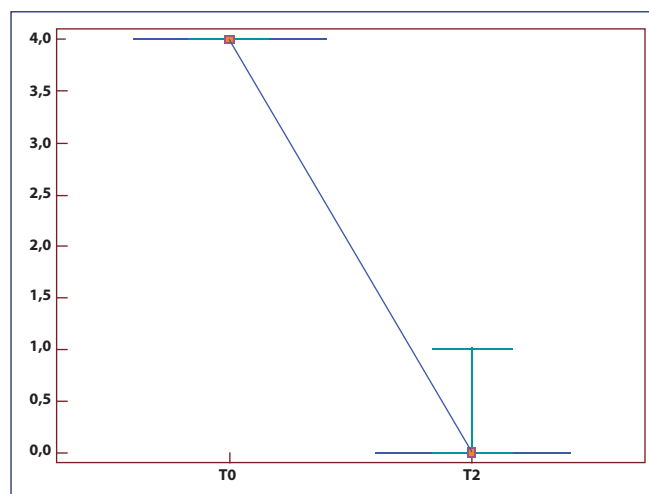


Figure 1. Trend of variation of median number of GW between treatment sessions.

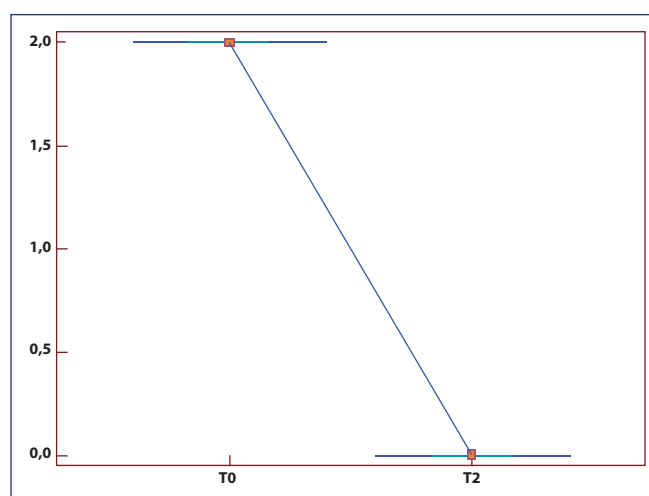


Figure 2. Trend of variation of median number of quadrants involved between treatment sessions.

sexually transmitted infections (2-4, 24), although the presence of national vaccination programs.

Various highly aggressive methods have been used for treatment of GW but can lead to sequelae like scars and deformations with a high recurrence rate (25) and can be associated with pain and HPV transmission, if HPV particles are present in the smoke plume produced by destructive therapies (26). Therefore, topical therapies offer some advantages as they can be used both by doctors and by patients at home and they can be useful for treatment of multiple lesions, which have a substantial risk of latent HPV presence in the clinically normal epithelium beyond the warts (26).

Topically applied therapeutic agents for external genital warts include imiquimod, podophyllotoxin, sinecatechins, trichloroacetic acid (TCA) and podophyllin and unconventional or newly emerging modalities, such as cidofovir gel, idoxuridine, polyhexamethylene biguanide, sodium nitrite with citric acid and SB206 12% (27).

The use of preparations based on *P. acnes* has been studied recently for treatment in skin warts (28), but there are not data for use in genital warts. Administered parentally, these preparations are highly immunogenic and are able to induce specific antibodies (29, 30) for immune response to HPV infection. There are no literature data to support the immunostimulating effect of topical bacterial lysate preparations, but, *in vitro*, preliminary studies have suggested an antioxidant activity of the *P. acnes* preparation subject of this study (data not shown), a well-recognized mechanism that results in the reduction of inflammatory process (31) and the resolution of the tissue damage (32).

The aim of the present study was to evaluate the grade of satisfaction, the local tolerability, and the effect on the number of GW and the number of quadrants involved in patients waiting for destructive therapy treated with *P. acnes* preparations. Our data showed a high satisfaction grade (discreet, good or excellent) after 30 and 60 days of topic application, without a significative variation during the study period. No incidence of side effects and a significative improvement in local tolerability after 30 and 60 days with high rates of patients with no symptoms (erythema or barely noticeable erythema) throughout the treatment period (85% and 96.6% after 30 and 60 days of treatment, respectively) was recorded. No patient decided to undergo destructive treatment after 30 days of therapy even in cases where there was a partial or absent re-

sponse. This may be due to a decrease in the symptoms perceived by the patients, to the absence of side effects and in most of the patients of the study, to the reduction of the number of warts. The gel formulation relieves itching and burning localized in the ano-genital area with genital warts; very often condylomatosis can be symptomatic at the time of diagnosis (20-23). Only two cases reported the onset of a slight erythema without edema after 60 days of therapy, without however determining the interruption of the treatment.

This level of tolerability is certainly higher than that reported in a recent meta-analysis (27), where many of the topical drugs used determined severe adverse events that led to patient withdrawal: the analysis of 17 studies showed that imiquimod 5% cream (OR: 8.68; 95% CI: 1.01-74.43), podophyllin 2.0% solution (OR: 38.43; 95% CI: 1.28-1156.07), podophyllotoxin 0.5% cream (OR: 5.98; 95% CI: 1.07-33.54), polyhexamethylene biguanide cream (OR: 55.87; 95% CI: 3.33-937.61) and sinecatechins 10% (OR: 8.03; 95% CI: 3.97-16.24) and 15% cream (OR: 8.54; 95% CI: 4.23-17.25) were associated with significantly higher numbers of patients with severe adverse events or patients who were lost to follow-up because of treatment-related side effects, compared to the placebo; Imiquimod 5% cream can determine the development of severe erythema and erosions even in the 40% of cases, podophyllin 2.0%, polyhexamethylene biguanide cream, and sinecatechins seems to determine severe local reaction or severe erythema in 4, 22 and 28% of cases, respectively.

The analysis of the rate of persistence or regression of GW after 60 days of therapy showed a significant decrease in the median number of GW and of quadrants involved and a complete response to therapy in 61.6% of patients that, therefore, they avoided a destructive therapy. This data is not comparable with other topical preparations for GW (27) because there is not a cohort treated with placebo and because, despite the number of our treated patients with *P. acnes* preparations is considerable, it would be necessary an efficacy studies or a RCT to evaluate their outcomes. When compared to placebo (25), all other treatments were significantly more efficacious: Podophyllotoxin 0.5% solution was significantly superior to imiquimod 5% cream for lesion clearance, although it was associated with a higher overall rate of adverse events. Sinecatechins were inferior to imiquimod 5% cream in wart clearance. For recurrence, all modalities did

not significantly differ from each other. Some unconventional agents were potentially better than conventional therapies regarding their efficacy or safety, although additional studies are required to confirm these results.

This study is not without limitations: the absence of a control group does not allow a real evaluation of efficacy but only of tolerability and grade of satisfaction. Moreover, among the 23 patients with partial or absent response after 60 days of therapy, 20 were subjected to destructive therapy, not allowing the evaluation of any subsequent spontaneous complete remission, as approximately 30 percent of all warts could regress within the first four months of infection (8). However, the homogeneity of the study cohort enrolled allow to consider assessable the grade of satisfaction of the patients. Immunoderm® could be then useful in patients who would like not to be subject to destructive treatments with related pain and risk of sequelae. Moreover, data available showed a long-term absence of relapse of GW (0 cases at T₃ and T₄ follow-up), also considering that most of GW will recur within three months of infection, even after undergoing appropriate treatments (9).

The real prevention of GW can be achieved by the use of the quadrivalent or nonavalent prophylactic HPV vaccines administered prior to sexual debut, as well as the meticulous use of condoms (1). Where coverage of the quadrivalent vaccine has been high, marked reductions in GW are being seen in young women of vaccine-eligible age, as well as in young males (1, 5).

A prospective single-center study (33) reported that recurrence for high grade cervical intraepithelial neoplasia (CIN2+) occurred in 6.4% of unvaccinated women and 1.2% of vaccinated women 30 days post LEEP. The effectiveness of vaccination in reducing recurrence after treatment of warts

should be assessed, also in association with immune stimulants substances.

P. acnes preparation has shown a high tolerability and a high grade of satisfaction in patients waiting for destructive treatment for GW. Moreover, even if this study does not allow conclusions regarding efficacy, all patients included decided to wait at least 60 days before being subjected to any destructive treatment, which was then necessary only in 31.8% of cases. A further study could evaluate its usefulness after destructive treatment to improve the symptoms experienced by patients.

According to literature, the uses of preparations based on *P. acnes* has been studied recently for treatment in skin warts (28), but there are not data for use in genital warts. Applied intradermally, these preparations resulted highly immunogenic and were able to induce specific antibodies (29, 30) for immune response to HPV infection. Nevertheless, the specific immunomodulatory properties of Immunoderm® are unpredictable, since there are still no evidences on the ability of such *P. acnes* preparation to penetrate the skin and acting as an immunomodulator.

However, the results obtained with this preliminary study enable to indicate the therapy with Immunoderm®, as possible innovative option in the treatment of GW. A prospective blinded RCT will be necessary to evaluate the *P. acnes* preparation efficacy in the treatment of condylomatosis. Currently, these results not allow a real evaluation of efficacy but show a good tolerability and acceptability profile of the Immunoderm®.

CONFLICTS OF INTERESTS

The authors declare that they have no conflict of interests.

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Incidence and risks of caesarean section in women aged ≥ 40 years

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ABSTRACT

Objective. Evaluating the rates of childbirth in women over 40 years of age in Tuscany (Italy) outlining the indications for caesarean section in this parturient population.

Methods. 227,871 women who delivered in Tuscany from 2011 to 2018 using data of Birth Assistance Certificate linked with hospital discharge registry were observed. Caesarean section indications were reported as dismissal diagnoses. Logistic models (adjusted for parity, ART and BMI) were carried out for the maternal age risk factor.

Results. The caesarean section rate increases significantly with age. Multivariate analysis confirmed that women over 40 years of age have a higher risk of a caesarean section due to pathologies such as diabetes or eclampsia which are clearly more frequent in these categories of women. Furthermore, the data shows that the caesarean section in some cases was carried out due solely to the age of a primiparous woman.

Conclusions. The phenomenon affects health services and social costs and should make us reflect upon the underlying reasons that bring women to delay their reproductive project and where necessary implement appropriate political strategies.

SOMMARIO

Scopo. Valutare il parto nelle donne over 40 in Toscana e descrivere le indicazioni al taglio cesareo in questa popolazione.

Metodi. Da Certificato di Assistenza al Parto linkato con la SDO sono state estratte 227.871 donne che hanno partorito in Toscana tra il 2011 e il 2018. Attraverso le diagnosi di dimissione è stato possibile identificare le principali indicazioni al cesareo. Per queste sono stati effettuati dei modelli logistici (univariati e multivariati aggiustati per parità, PMA e BMI) per il fattore di rischio età materna.

Risultati. Aumenta significativamente all'aumentare dell'età il ricorso al taglio cesareo, soprattutto quello di elezione e quello in urgenza, a testimonianza di una maggiore incidenza di condizioni patologiche. L'analisi multivariata conferma per le over 40 un rischio maggiore di taglio cesareo a causa di patologie come il diabete o l'eclampsia che risultano chiaramente più frequenti in queste categorie di donne. I dati mostrano inoltre che in alcuni casi il cesareo viene effettuato solo per indicazione materna.

Conclusioni. Questo incide sui servizi sanitari e sui costi sociali e dovrebbe fare riflettere sulle motivazioni che conducono le donne a ritardare il loro progetto riproduttivo mettendo in atto scelte politiche conseguenti e adeguate.

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Key words:

Pregnancy; caesarian section; diabetes; eclampsia; maternal indications; advanced maternal age.

INTRODUCTION

In recent decades, there has been a tendency for women in developed countries to postpone their reproductive plans to an older age (1-3). The phenomenon is multifactorial and a combination of medical, cultural and social components (4-6): contraceptive methods availability; achievement of higher levels of education by women; entrance of women into typically male working environments where maternity is not sufficiently supported. Furthermore, cultural changes that make women not feel ready to have a child at a young age and lack of employment policies and social networks to support working women may also play a role. Other circumstances are represented by economic instability in relationships, and the availability of assisted reproduction treatments. With adequate prenatal care it is possible for older women to have successful pregnancies with a generally favourable outcome comparable to that of young women. Most women who have a single spontaneous pregnancy at 43 or older have a successful pregnancy outcome. However, compared to women of younger age, they have significantly higher risks of caesarean section and preterm birth.

These risks increase significantly with *in vitro* fertilization and twin pregnancy among older women (1). The advanced maternal age is characterized by a greater risk of complications of maternal pregnancy such as hypertension, diabetes, and placental problems (7-9). According to Eurostat data (10), Italy holds the European record of women with the first child at 40 years of age (in 2017, 10.5% of women with the first child was at least 40 years old compared to the European average of 6%) and is one of the first countries in Europe where more than half of women conceive their first child after age 30. Tuscany is ranked sixth among the Italian regions for the largest proportion of over 40 childbirth (11). The aim of this study is to evaluate the phenomenon of childbirth in over 40 women in Tuscany and to describe in particular the reasons for caesarean sections in this population of parturients.

MATERIALS AND METHODS

The data sources used for the study were the Childbirth Assistance Certificate (Cedap) and the Nosological Card (SDO). We extracted from Cedap the women who gave birth in Tuscany in the period

2011-2018. A procedure of record linkage was carried out between the two archives Cedap and SDO to identify hospital admissions for giving birth. The record-linkage procedure was carried out using as the first key field the number of the clinical record present in the SDO archive, year of practice and birth-point code or as a second key field the general identification information of the woman. SDO allows identifying the primary discharge diagnosis and five additional diagnoses coded according to the International Classification of Diseases, ninth review (ICD-9CM). Through the dismissal diagnoses after birth it was possible to identify the main indications for caesarean section: Previous c-section, Podalic fetus/abnormal position, Fetal stress, Multiple pregnancy, Dystocia, Failed induction, IUGR, Placental abruption, Diabetes, Eclampsia, Placenta abnormalities and Maternal indications. The codes that led to the classification of the caesarean section indications are reported in **table I**.

The analysis is divided by age of the woman: the two groups 40-42 years and 43 years and more in which the over 40 population is extracted are compared with women under 40 years of age and with the average for Tuscany. In descriptive analyses frequency distributions and the χ^2 test for comparison between the 3 age classes into which the study population is divided were used. Results were considered significant when $p < 0.05$.

Twelve individual multivariate logistic models using each of the CS indications as an outcome and age, parity, ART and BMI as independent variables were carried out. The 95% odds ratios and confidence intervals (CI) were calculated.

Statistical analysis was carried out with the statistical package STATA/SE, version 14.0.

RESULTS

During the study period the number of women over 40 who gave birth in Tuscany was 20214 (8.9% of all births that took place in the region): 7.4% in 2011, reaching 9.8% in 2018. At the same time, 4800 women were aged over 43 years at the moment of child-birth (2.1%).

Thirty-six percent of the 40-42-years old experienced their first birth and adding the women aged 43 and over the rate rises to 46.7%. These women are employed in a higher proportion than the younger ones and have a higher educational qualification. The proportion of foreign mothers in the

Table I. Codes used to classify the main indication for caesarean section.

| Indication for caesarean section | Code (ICD-9CM) |
|----------------------------------|--|
| Previous c-section | 65420, 65421, 65423 |
| Podalic fetus/abnormal position | 65201, 65203, 65210, 65211, 65213, 65220, 65221, 65223, 65231, 65233, 65241, 65243, 65251, 65253, 65283, 65291, 65441, 65442, 66001, 6961 |
| Fetal stress | 64403, 64410, 64420, 65371, 65451, 65501, 65531, 65541, 65571, 65581, 65591, 65601, 65611, 65621, 65630, 65631, 65633, 65641, 65681, 65841, 65970, 65971, 65973, 66131, 66141, 66300, 66301, 66310, 66311, 66313, 66321, 66323, 66341, 66380, 66381, 66980, 7424 |
| Multiple pregnancy | 65100, 65101, 65103, 65111, 65121, 65131, 65141, 65161, 65171, 65181, 65191, 65193, 65261, 66051, V272 |
| Dystocia | 2189, 65271, 65301, 65341, 65343, 65350, 65351, 65353, 65361, 65381, 65391, 65411, 65412, 65413, 65461, 65471, 65481, 65661, 65663, 65703, 65830, 65831, 66000, 66003, 66010, 66011, 66013, 66020, 66021, 66030, 66031, 66033, 66040, 66041, 66071, 66080, 66081, 66083, 66090, 66091, 66093, 66140, 66143, 66190, 66191, 66200, 66201, 66203, 66210, 66211, 66213, 66220, 66221, 66223 |
| Failed induction | 64510, 64511, 64513, 64520, 64521, 64523, 65810, 65813, 65820, 65821, 65823, 65900, 65901, 65910, 65911, 65913, 66060, 66061, 66063, 66100, 66103, 66110, 66111, 66121, 66123 |
| IUGR | 65650, 65651, 65653, 65691, 76490 |
| Placental abruption | 64121, 64123 |
| Diabetes | 64800, 64801, 64802, 64803, 64881, 64882, 64883, 64884 |
| Eclampsia | 64091, 64200, 64201, 64202, 64203, 64212, 64213, 64221, 64223, 64231, 64232, 64233, 64241, 64242, 64251, 64252, 64254, 64260, 64261, 64262, 64271, 64291, 64292, 64293, 64294, 64611, 64613, 64671, 66920, 66921 |
| Placenta abnormalities | 6266, 64081, 64101, 64103, 64110, 64111, 64113, 64131, 64181, 64191, 64211, 64230, 64243, 64253, 64263, 64673, 65281, 65671, 66351, 66602 |
| Maternal indications | 389, 42, 5410, 7811, 7950, 1715, 1745, 1888, 1889, 1890, 220, 2375, 2382, 2449, 2452, 2699, 29544, 29622, 30000, 30020, 30029, 30562, 3080, 3310, 3312, 34201, 3499, 3510, 3619, 36210, 3671, 3870, 41071, 4111, 4211, 4270, 4281, 42821, 43401, 4467, 4549, 462, 4660, 485, 49121, 5118, 5185, 51881, 51884, 53101, 53500, 55090, 566, 56881, 5693, 56983, 5770, 5789, 5859, 591, 5921, 6183, 62211, 6288, 6390, 64321, 64621, 64631, 64641, 64661, 64662, 64701, 64721, 64731, 64741, 64761, 64762, 64781, 64783, 64791, 64793, 64794, 64811, 64812, 64821, 64822, 64823, 64831, 64841, 64843, 64851, 64852, 64861, 64862, 64871, 64872, 64873, 64911, 64914, 64921, 64931, 64941, 64942, 65311, 65321, 65331, 65401, 65403, 65460, 65462, 65463, 65521, 65920, 65921, 65931, 65950, 65951, 65961, 66491, 66561, 66632, 66801, 66811, 66821, 66881, 66883, 66900, 66901, 66902, 66903, 66910, 66911, 66912, 66923, 67111, 67122, 67131, 67191, 67202, 67204, 67301, 67401, 67410, 67451, 67452, 74781, 75249, 7566, 7802S, 7806, 78551, 78559, 78650, 7880, 78900, 81201, 81340, 82020, 86519, 9051, 9896, V1249, V261, V580S, V5811, V581, V621, V719 |

over 40 age group is significantly lower than that recorded in the overall total of mothers giving birth in Tuscany where this percentage is 27.6%.

Mothers over 40 years are slightly more overweight or obese when compared to younger mothers. Of the 40-42 years old 34.0% and 39.9% of the over 43 year age group had at least one miscarriage before giving birth compared to 17.8% of women aged under 40 years of age.

In the group of 40-42 years old and the group over 43 years 7.3% and 25.5 % respectively have used ART compared to 2.1% in the group of women aged under 40 years of age.

Pathological pregnancy occurred in 38.3% of the women 43 years or older compared to 27.9% of the 40-42 years old, and 19.9% of the women under 40 years of age.

The proportion of multiple births increases in women aged 40-42 compared to younger women and reaches 7.4% of the births in women over 43 years of age, also due to the greater use of ART. As a consequence of this, the women over 40 years old and especially those over 43 years of age give birth

to a greater share of preterm infants (< 37 weeks): 8.2% and 14.4% compared to the 6.2% that occurs in the group of women under 40.

The practise of caesarean section increases significantly with age (25.0%, 38.6% and 56.1% respectively in the three age groups considered), above all in the categories of both elective and urgency, reflecting a higher incidence of pathological conditions (**table II**).

The frequency distribution of the caesarean section indications classes was: previous CS (29.8%), podalic fetus/abnormal position (14.5%), fetal stress (13.6%), dystocia (11.1%), failed induction (5.9%), multiple pregnancy (5.0%), eclampsia (3.4%), maternal indications (2.6%), placenta abnormalities (2.5%), IUGR (2.2%), placental abruption (1.7%) and diabetes (1.5%).

In the women over 40 years of age, but especially those older than 43 years, pathologies such as eclampsia and diabetes (**table III**) emerge in a greater proportion when compared to the younger women. It is observed that in 7.8% of the cases the caesarean is carried out for maternal indication in

Table II. Socio-demographic characteristics of women who delivery in Tuscany by age groups (% , p-value) and comparison with the average regional data. Years 2011-2018.

| Variabile | Age of the woman | | | p-value* | Total |
|--|------------------|-------|------|----------|--------|
| | < 40 | 40-42 | ≥ 43 | | |
| Number of women | 20765 | 15414 | 4800 | | 227871 |
| Primiparous (%) | 53.1 | 36.4 | 46.7 | < 0.0000 | 51.9 |
| Unmarried (%) | 39.5 | 35.8 | 37.0 | < 0.0000 | 39.2 |
| Employed (%) | 63.4 | 77.6 | 76.9 | < 0.0000 | 64.6 |
| Foreigners (%) | 29.2 | 12.0 | 10.9 | < 0.0000 | 27.6 |
| No academic qualifications or primary school (%) | 27.6 | 19.4 | 20.6 | < 0.0000 | 26.9 |
| Previuos miscarriages (%) | 17.8 | 34.0 | 39.9 | < 0.0000 | 19.4 |
| Obesity/overweight (%) | 22.2 | 24.5 | 25.5 | < 0.0000 | 22.4 |
| ART use (%) | 2.1 | 7.3 | 25.5 | < 0.0000 | 2.9 |
| Pathological pregnancy (%) | 19.9 | 27.9 | 38.3 | < 0.0000 | 20.8 |
| Elective CS (%) | 12.2 | 22.3 | 35.2 | < 0.0000 | 13.4 |
| CS during labour (%) | 7.6 | 8.4 | 8.7 | < 0.0000 | 7.7 |
| Urgen CS (%) | 5.2 | 7.9 | 12.2 | < 0.0000 | 5.5 |
| Multiple birth (%) | 1.7 | 2.5 | 7.4 | < 0.0000 | 1.8 |
| Preterm birth (< 37 wks GA) (%) | 6.2 | 8.2 | 14.4 | < 0.0000 | 6.5 |

*The p value refers to the three age groups.

Table III. Caesarean section indications in women who delivery in Tuscany with caesarean section by age groups (% , p-value) and comparison with the regional average data. Years 2011-2018.

| | Caesarean section deliveries | | | | | Caesarean deliveries Primiparous and single birth | | | | |
|---------------------------------|------------------------------|-------|------|----------|-------|---|-------|------|----------|-------|
| | Age of the woman | | | | Total | Age of the woman | | | | Total |
| | < 40 | 40-42 | ≥ 43 | p-value* | | < 40 | 40-42 | ≥ 43 | p-value* | |
| Number of mothers with CS | 48282 | 5464 | 2373 | | 56119 | 22632 | 1926 | 1016 | 25574 | 25574 |
| Previous c-section | 29.5 | 35.3 | 23.2 | < 0.0000 | 29.8 | - | - | - | - | - |
| Podalic fetus/abnormal position | 15.0 | 12.3 | 11.0 | < 0.0000 | 14.5 | 22.1 | 19.8 | 15.1 | < 0.0000 | 21.7 |
| Fetal stress | 14.1 | 10.9 | 9.9 | < 0.0000 | 13.6 | 22.1 | 18.1 | 13.7 | < 0.0000 | 21.4 |
| Dystocia | 11.6 | 9.0 | 6.7 | < 0.0000 | 11.1 | 18.7 | 15.5 | 9.8 | < 0.0000 | 18.1 |
| Failed induction | 5.9 | 5.4 | 6.7 | 0.098 | 5.9 | 10.9 | 12.5 | 12.3 | 0.115 | 11.0 |
| Multiple pregnancy | 4.7 | 5.3 | 11.4 | < 0.0000 | 5.0 | - | - | - | | - |
| Eclampsia | 3.2 | 4.1 | 6.2 | < 0.0000 | 3.4 | 4.5 | 7.1 | 9.9 | < 0.0000 | 4.9 |
| Maternal indications | 2.3 | 3.1 | 7.8 | < 0.0000 | 2.6 | 3.5 | 5.6 | 14.6 | < 0.0000 | 4.1 |
| Placenta abnormalities | 2.3 | 3.2 | 3.1 | < 0.0000 | 2.5 | 2.7 | 4.0 | 3.6 | 0.003 | 2.8 |
| IUGR | 2.2 | 2.2 | 2.9 | 0.07 | 2.2 | 3.3 | 3.7 | 4.0 | 0.362 | 3.3 |
| Placental abruption | 1.7 | 1.5 | 1.1 | 0.04 | 1.7 | 2.1 | 2.2 | 1.4 | 0.22 | 2.1 |
| Diabetes | 1.4 | 1.8 | 3.1 | < 0.0000 | 1.5 | 1.7 | 2.9 | 5.0 | < 0.0000 | 2.0 |
| Other | 6.1 | 6.0 | 6.9 | 0.256 | 6.2 | 8.4 | 8.5 | 10.5 | 0.083 | 8.5 |

*The p value refers to the three age groups.

the over 43s and the rate rises to 14.6% if only single-birth primiparous women are considered.

As expected, the incidence of caesarean section increases with increasing maternal age for all CS indications, but especially for pathologies such as eclampsia and diabetes (table IV).

The multivariate logistic models on these 12 variables, adjusted for parity, BMI and ART show that while younger women have a higher risk of

having caesarean section following fetal stress or twin pregnancy, for women aged 40-42 and over 43 years old there is a greater risk for pathologies such as eclampsia and diabetes, a risk that increases significantly with increasing age. Women aged over 43 have a more than one and a half times greater risk of having caesarean section due to eclampsia (OR: 1.62; IC 95%: 1.33-1.98) than women younger than 40 years and a more than double risk

Table IV. Incidence (%) and risk of caesarean section by each CS indications with the age as risk factor. Years 2011-2018 (OR: IC 95%).

| Indication for caesarean section | < 40 | | | 40-42 | | | ≥ 43 | | |
|----------------------------------|------|------|----------|-------|------|-------------|-------|------|-------------|
| | % | OR # | (IC 95%) | % | OR # | (IC 95%) | % | OR # | (IC 95%) |
| Previous c-section (%) | 91.9 | 1.00 | - | 94.4 | 1.01 | (0.93-1.09) | 97.2 | 0.82 | (0.72-0.94) |
| Podalic fetus/ abnormal position | 98.1 | 1.00 | - | 97.7 | 0.94 | (0.86-1.03) | 98.9 | 0.83 | (0.72-0.95) |
| Fetal stress | 72.0 | 1.00 | - | 79.0 | 0.89 | (0.81-0.97) | 87.7 | 0.83 | (0.72-0.97) |
| Dystocia | 68.8 | 1.00 | - | 76.6 | 0.91 | (0.82-1.00) | 83.2 | 0.67 | (0.57-0.80) |
| Failed induction | 26.6 | 1.00 | - | 39.2 | 1.13 | (0.99-1.29) | 55.2 | 1.16 | (0.97-1.40) |
| Multiple pregnancy | 86.7 | 1.00 | - | 94.7 | 0.74 | (0.64-0.85) | 98.2 | 0.74 | (0.63-0.86) |
| Eclampsia | 39.5 | 1.00 | - | 55.9 | 1.33 | (1.14-1.56) | 70.7 | 1.62 | (1.33-1.98) |
| Maternal indications | 31.3 | 1.00 | - | 43.1 | 1.54 | (1.30-1.83) | 74.9 | 3.52 | (2.93-4.23) |
| Placenta abnormalities | 77.9 | 1.00 | - | 87.1 | 1.40 | (1.18-1.65) | 88.1 | 1.25 | (0.97-1.62) |
| IUGR | 45.3 | 1.00 | - | 59.8 | 1.04 | (0.84-1.28) | 80.3 | 1.21 | (0.92-1.59) |
| Placental abruption | 98.1 | 1.00 | - | 98.8 | 0.89 | (0.69-1.13) | 100.0 | 0.72 | (0.47-1.09) |
| Diabetes | 14.1 | 1.00 | - | 19.1 | 1.27 | (1.01-1.60) | 41.1 | 2.38 | (1.83-3.11) |

Adjusted for parity, ART and BMI; reference age category: < 40 years.

(OR: 2.38; IC 95%: 1.83-3.11) for diabetes. In addition, the maternal caesarean section indication is three and a half times more likely in women older than 43 years compared to the ones younger than 40 years (table IV).

DISCUSSION

Our data confirm the increased incidence of women aged over 40 years in Tuscany over the past 10 years (12). It is a population that most frequently turns to ART with a consequent greater frequency of multiple pregnancies and pregnancies at risk.

As it is also reported in other studies, there is a greater incidence of obstetric complications or medical conditions such as hypertension and diabetes (13, 14) for these women and especially for women over 43 years of age, risks intensified with increasing maternal age.

The greater recourse to medically assisted reproductive techniques and the greater incidence of maternal pathologies means that women over 40 years have a higher risk of caesarean section compared to those under 40 years (15-19). Above all,

the data show a higher frequency of emergency caesarean section in the group over 40 years as evidence of a greater incidence of pathological conditions (20). The over 40s in fact have a higher risk of a caesarean section due to pathologies such as diabetes or eclampsia which are clearly more frequent in these categories of women. Furthermore, the data show that the caesarean section could be carried out for maternal indication if the woman is an elderly primiparous woman (21-27).

The risks of caesarean section delivery, in addition to those immediate for maternal morbidity during childbirth, are also associated with a doubling of the risk of neonatal mortality (28).

This affects health services and social costs and should make us reflect upon the underlying reasons that bring women to delay their reproductive project and where necessary implement appropriate political strategies.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Validation of the performance of “Fast Lung Ultrasound Teaching Program” for gynecologists/obstetricians dealing with pregnant women with suspicion of COVID-19 infection: an Italian prospective multicenter study

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ABSTRACT

Objective. The goal of this study was to validate a “fast lung ultrasound teaching program” in a large number of Italian gynaecologists/obstetricians skilled on ultrasound, by comparing the number of correct answers in pre and post-training tests.

Methods. This is a prospective, multicenter study. Gynaecologic consultants, who are member of two Italian Societies – SIGO and SIEOG, with at least 5 years of experience in ultrasound, were invited to participate. The fast lung teaching program was available online and consisted of three phases: 1) a pre-test with 10 ultrasound video-clips, 2) a 40 minutes theoretical course, 3) a post-test with the administration of the same 10 ultrasound video-clips, showed in a different order. The results of the pre-test were compared with those of post-test analysis.

Results. 108 gynaecologists/obstetricians completed the program. Considering pre-test results, the median number of correct answers was 7 (range 2-10). In particular, 55 of them (50.9%) had a number of correct answers equal or less than 7/10 (“inadequate pre-test group”), whereas 53/108 (49.1%) had a number of correct answers equal or more than 8/10. Considering post-test analysis of all trainees, the median number of correct answers was 9 (range 6-10), with a mean dif-

SOMMARIO

Obiettivo. L’obiettivo di questo studio è stato quello di convalidare un “programma di insegnamento dell’ecografia polmonare veloce” in un gran numero di ginecologi/ostetrici italiani esperti in ultrasuoni, confrontando il numero di risposte corrette nei test pre e post allenamento.

Metodi. Questo è uno studio prospettico multicentrico. Sono stati invitati a partecipare consulenti ginecologici, membri di due Società italiane – SIGO e SIEOG, con almeno 5 anni di esperienza nell’ecografia. Il programma di insegnamento del polmone veloce era disponibile online e consisteva in tre fasi: 1) un pre-test con 10 videoclip ecografici, 2) un corso teorico di 40 minuti, 3) un post-test con somministrazione degli stessi 10 video ecografici -clips, mostrati in un ordine diverso. I risultati del pre-test sono stati confrontati con quelli dell’analisi post-test.

Risultati. 108 ginecologi/ostetrici hanno completato il programma. Considerando i risultati pre-test, il numero medio di risposte corrette era 7 (range 2-10). In particolare, 55 di loro (50.9%) avevano un numero di risposte corrette uguale o inferiore a 7/10 (“gruppo pre-test inadeguato”), mentre 53/108 (49.1%) avevano un numero di risposte corrette uguale o superiore di 8/10. Considerando l’analisi post-test di tutti i partecipanti, il numero medio di risposte corrette è stato 9 (range 6-10), con una differenza media tra pre-test e post-test pari a

ference between pre-test and post-test equal to 1.9 points and a median difference of 2 points. When considering only the “inadequate pre-test group”, the median number of correct responses at pre-test analysis was 6 (range 2-7), and at post-test analysis was 9 (range 6-10).

Conclusions. The “fast lung ultrasound teaching program” was able to offer basic theoretical principles of lung ultrasound to gynaecologists/obstetricians. The consultants with inadequate pre-test results were also able to obtain a satisfactory result after the program.

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1.9 punti e una differenza mediana di 2 punti. Quando si considera solo il “gruppo pre-test inadeguato”, il numero mediano di risposte corrette all’analisi pre-test era 6 (range 2-7) e all’analisi post-test era 9 (range 6-10).

Conclusioni. Il “programma di insegnamento dell’ecografia polmonare veloce” è stato in grado di offrire i principi teorici di base dell’ecografia polmonare a ginecologi/ostetrici. I consulenti con risultati pre-test inadeguati sono stati anche in grado di ottenere un risultato soddisfacente dopo il programma.

Key words:

Lung ultrasound; pregnancy; COVID-19; teaching; Gynaecology.

INTRODUCTION

Lung ultrasound has newly been suggested as an imaging method to detect lung involvement in patients affected by COVID-19 (1-3). Although chest Computer Tomography (CT) is the most accurate technique in diagnosis chest involvement in patients with COVID-19 pneumonia (4) lung ultrasound can be a valid tool in the diagnostic path of this pathology, with some advantages (5). Indeed, the low cost, the large availability, and the possibility to perform the examination at the bed site of the patients, make this diagnostic method attractive in this health emergency (6-8).

Several efforts are being made to contain the Covid-19 pandemic also in the Gynaecological and obstetrical field (9, 10). To this regards, the International Society of Ultrasound in Obstetrics and Gynaecology (ISUOG) has fixed the potential utility of lung ultrasound examination in pregnant women and recently published an “how to” practical approach aimed at alerting and preparing gynaecologists/obstetricians, already skilled on ultrasound, to examine the lung of pregnant women with suspected COVID-19 (11). Moreover, we recently develop a “fast lung ultrasound teaching program” providing good results for the acquisition of theoretical skills in a small group of obstetricians/gynecologists of our Institution (12). In the present study, we aim at validating the “fast lung ultrasound teaching program” in a large number of Italian gynaecologists/obstetricians skilled on ultrasound.

MATERIALS AND METHODS

This is a prospective, interventional, multicenter study, performed at Fondazione Policlinico Universitario A. Gemelli, IRCCS, in Rome, Italy. The protocol was approved by Ethical Committee (Prot. ID 30-87) on 2nd April 2020. The study aims at validating a fast lung ultrasound teaching program in a large number of gynecologists/obstetricians by comparing the number of correct answers in pre and post-training tests. The “fast lung ultrasound teaching program” is freely available on the ICLUS platform (<https://covid19.disi.unitn.it/iclusdb/login>). To obtain access to the platform please send a request to libertario.demi@unitn.it.

Gynaecologic consultants, who are member of two Italian Societies – SIGO (Società Italiana di Ginecologia e Ostetricia) and SIEOG (Società Italiana di Ecografia in Ostetricia e Ginecologia), with at least 5 years of experience in gynaecologic or obstetric ultrasound examination, were invited to participate. All the participants signed a written consent form. Demographic characteristics including age, type of hospital, years of experience on ultrasound were recorded.

The fast lung teaching program consisted of three phases. Phase 1 of 15 minutes pre-test, with 10 ultrasound video-clips, collected by G.S., R.C., A.S., R.I. Each video showed normal or pathological typical ultrasound patterns. After watching the video-clip, each learner had to answer about the pattern, choosing among 6 possibilities a) normal; b) clear and distinct vertical artifacts with small pleu-

ral alterations (score 1) or broken pleural line and small consolidations (score 2); c) white lung without evident subpleural consolidations (score 3); d) white lung with evident subpleural consolidations (score 3); e) pattern not compatible (vast consolidations); f) pattern not compatible (pleural effusions) (2). Phase 2 consisting of 40 minutes theoretical course, provided by two pneumologists with high expertise in lung ultrasonography (A.S. and R.I.), addressing clinical and ultrasound issues related to lung ultrasound. It included a first section on physical basic principles of the interaction between the ultrasound beam and the lung. A second section described the main semeiotic of lung ultrasound patterns. A third section included some data about the clinical meaning suggestive of lung patterns. A fourth section dedicated to the presentation of lung ultrasound patterns indicative of COVID-19 pneumonia. Phase 3 of 15 minutes post-test with the administration of the same 10 ultrasound video-clips of the phase 1, showed in a different order.

Sample size and statistical analysis

Given the primary objective of the study and given the lack of previous data on this topic, we proposed a sample size of $N = 102$ subject. This dimension is appropriate to detect a mean of paired differences ranging from 1 to 10, with a standard deviation of paired differences equal to 3 with a power ranging from 90% to 98%.

The results of the pre- test were compared with those of post-test analysis for each examiner.

Moreover, a second analysis including only those trainees with a number of correct answers equal or less than 7/10 (inadequate pre-test group) was performed. Results, expressed as number of correct answers, was summarized with median, minimum and maximum value. The Wilcoxon signed-rank test was used to determine whether there is a median difference between paired observations. Two-sided test was used and the significance level was set at $\alpha = 0.05$.

Statistical analyses were performed using the Statistical Package for the Social Sciences software (SPSS v. 25).

RESULTS

One hundred and twenty gynecologists/obstetricians attended the training program and 108 of them completed the program, 84 females (77.8%) and 24

males (22.2%). Demographic details of trainees are presented in **table I**. Most participants were in the 30-40 age group (50/108, 46.3%), and living in the central Italian region (52/108, 48.1%). Most trainees had a previous experience of 5-10 years (43/108, 39.8%) on ultrasound. The vast majority of participants were working at a regional hospital (54/108, 50.0%) or at university hospital (37/108, 34.3%).

Considering pre-test results, the median number of correct answers was 7 (range 2-10). In particular, 55 of them (50.9%) had a number of correct answers equal or less than 7/10 ("inadequate pre-test group"), whereas 53/108 (49.1%) had a number of correct answers equal or more than 8/10.

Considering post-test analysis of all trainees, the median number of correct answers was 9 (range 6-10), with a mean difference between pre-test and post-test equal to 1.9 points and a median difference of 2 points. On a total of 108 participants only 6 of them (5.5%) performed better in the pre-test than in the post-test, 13 (12%) maintained the same number of correct answers and 89 (82.4%) improved the performance result.

When considering only the "inadequate pre-test group", the median number of correct responses at pre-test analysis was 6 (range 2-7), and at post-test analysis was 9 (range 6-10).

Table I. Demographic characteristics of the trainees.

| Characteristics | Number (%) |
|---------------------------------------|------------|
| <i>Gender</i> | |
| F | 84 (77.8) |
| M | 24 (22.2) |
| <i>Age</i> | |
| 30-40 | 50 (46.3) |
| 41-50 | 34 (31.5) |
| 51-60 | 15 (13.9) |
| 61-70 | 9 (8.3) |
| <i>Years of ultrasound experience</i> | |
| 5-10 | 43 (39.8) |
| 11-20 | 40 (37.0) |
| > 21 | 25 (23.2) |
| <i>Specific areas of interest</i> | |
| Both obstetric and gynecology | 48 (44.4) |
| Prenatal diagnosis | 26 (24.1) |
| Gynaecologic oncology | 26 (24.1) |
| Gynecology | 8 (7.4) |
| <i>Type of Hospital</i> | |
| Regional Hospital | 54 (50.0) |
| University Hospital | 37 (34.3) |
| Private Clinic | 10 (9.2) |
| Other public Centers | 7 (6.5) |
| <i>Geographical area of Italy</i> | |
| North | 36 (33.3) |
| Center | 52 (48.1) |
| South | 12 (11.2) |
| Islands | 8 (7.4) |

The Wilcoxon signed-rank test highlighted a statistically significant median difference between paired observations, $p < 0.0005$.

DISCUSSION

In this study, we presented the efficacy of “fast lung ultrasound teaching program” designed for gynaecologists/obstetricians already skilled on ultrasound. We demonstrated that such program is able to offer basic theoretical principles of lung ultrasound to gynaecologists and obstetricians. Indeed, also those consultants with inadequate pre-test results were able to obtain a satisfactory result after the program.

To the best of our knowledge this is the first “fast lung ultrasound teaching program” on lung ultrasound for gynaecologists/obstetricians involved in the assistance of pregnant patients affected by COVID-19.

Our study has some limitations. First, this does not include a practical program, therefore the dynamic aspects of real-time ultrasound, such as patient compliance (*e.g.*, a dyspneic woman) has not been considered and could affect lung ultrasound performance. Secondly, difficult cases in terms of differential diagnosis between chronic pulmonary disease and COVID-19 pneumonia were not specifically addressed; this does not represent a huge bias as chronic lung diseases are not expected in pregnant women.

Although there is currently no general agreement on the best method to provide teaching program on lung ultrasound, our report confirmed previous data on the feasibility and effectiveness of such training (13). Other studies proposed lung ultrasound teaching programs, but most of them included a longer teaching time than that of our study and none of them were planned to teach gynaecologists (14-20). These studies focused on also teaching differential diagnosis of different thorax pathologies (*i.e.*, pericardial effusion, pneumothorax, and cardiac standstill) (14). On the contrary, we developed a model to recognize ultrasound signs of COVID-19 pneumonia in pregnant patients.

Considering both the efficacy of lung ultrasound in the detecting of COVID-19 pneumonia in pregnant

women, and the large number of gynaecologists/obstetricians in the world with high level of experience on ultrasound, the fast teaching program could be of clinical relevance in this emergency.

Indeed, at this critical time of pandemic, the possibility to learn how to perform lung ultrasound in a short time could be very useful in low resource countries where there is a lack of CT scan machines and the number of clinicians expert in diagnosis and management of pulmonary pathologies could be insufficient.

On the other hand, the possibility to learn how to perform lung ultrasound in a fast way could be very helpful also in high resource countries in which many hospitals could face a shortage of radiologists or available CT machines, as it happened in some areas. Moreover, the ability to examine lung with an ultrasound could help in triaging pregnant patients admitted to emergency for suspicion of COVID-19 (5, 7, 8). Point of care and the possibility to perform the examination at patient bed site make this diagnostic method of important value in health emergency and intensive care unit, avoiding to transfer critical patients. Finally, the possibility to increase the number of clinicians able to perform lung ultrasound other than pneumologists or radiologists, could help in the management of patients at domicile reducing the hospital load. This may be of great help in the second phase of the pandemic, especially in the follow up of positive patients.

CONCLUSIONS

In conclusion, our “fast lung ultrasound teaching program” is one possible model of implementing lung ultrasound education and it might be a useful launch pad for clinicians wishing to start using lung ultrasound in their clinical practice. At this critical point of pandemic, lung ultrasound can be of support in both low and high resource countries.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Dystocia in labour: diagnosis, management and culture of Italian midwives

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ABSTRACT

Objective. Dystocia in labour is the most common indication for primary caesarean sections. We have investigated how Italian midwives are informed and aware of the diagnosis of dystocia in labour, which strategies they implement and how their culture can affect clinical decisions.

Methods. Purpose-built questionnaire using convenience sampling on a voluntary basis. The research was carried out on a population of Italian midwives. The questionnaire was divided into three macro-areas: socio-demographic information; a clinical case with decision questions; operators' knowledge and clinical choices.

Results. 300 questionnaires were collected, and 289 were analysed. 60% of midwives would have not diagnosed active labour before 6 cm of dilation and would have adopted conservative management. 81% would adopt methods such as change of maternal posture, movement, and emotional support to solve dystocia rather than oxytocin and artificial rupture of membranes. 76% is aware that there is no single definition of dystocia, 80% do not know the definition of latent phase. The discussion on dystocia is rarely addressed in a context such as an audit.

Conclusions. Culture considered as experience, knowledge, and work context, could affect clinical practice. Most midwives showed interest in the subject by tackling it with a view that was mainly physiological. The need for training and structured discussion meetings is, in any case, important.

SOMMARIO

Obiettivo. La distocia in travaglio è la più comune indicazione al taglio cesareo primario. Abbiamo indagato quanto le ostetriche italiane siano informate e consapevoli della diagnosi di distocia in travaglio, quali strategie gestionali mettano in atto e quanto la loro cultura possa influenzare le decisioni cliniche.

Metodi. Indagine conoscitiva mediante questionario costruito *ad hoc* utilizzando un campionamento di convenienza su base volontaria. La ricerca è stata condotta su una popolazione di ostetriche. Il questionario è stato suddiviso in tre macro aree: informazioni socio-demografiche; caso clinico con domande decisionali; conoscenze degli operatori e loro scelte cliniche.

Risultati. Sono stati raccolti 300 questionari e ne sono stati analizzati 289. Il 60% delle ostetriche non avrebbe fatto diagnosi di travaglio attivo prima dei 6 cm di dilatazione e avrebbe adottato un management di attesa. L'81% adotterebbe metodi come il cambio di postura, il movimento e il supporto emotivo per la risoluzione della distocia a sfavore dell'ossitocina e dell'amnioressi. Il 76% è consapevole che non vi sia una definizione univoca di distocia, l'80% non conosce la definizione di fase latente. La discussione sulla distocia è raramente affrontata in un contesto come l'audit.

Conclusioni. La cultura intesa come esperienza, conoscenza e contesto lavorativo potrebbe influenzare la pratica clinica. La gran parte delle ostetriche ha mostrato interesse verso la tematica affrontandola in un'ottica prevalentemente di normalità. È comunque rilevante il bisogno di formazione e la necessità di incontri di discussione strutturati.

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Key words:

Pregnancy; labour dystocia; intrapartum strategy; cultural diversity; midwifery care; caesarean sections.

INTRODUCTION

Prolonged labour or dystocia is the main indication for interventions such as artificial rupture of membranes (AROM), oxytocin perfusion, vaginal operative deliveries, and urgent caesarean sections. The choice of our investigation arises, in particular, from the awareness of an objective fact: dystocia in labour is the most common indication for primary caesarean sections.

American data show that 34% – the largest share – of caesarean sections in United States has dystocia as a clinical indication (1). In Italy, dystocia is one of the four main indications for caesarean sections which, together with foetal distress, breech presentation and previous caesarean section, represent overall 70% of caesarean sections (2).

It is not possible at present to establish exactly the prevalence of dystocia, since both definition and diagnostic criteria are not univocal, and this is one of the problems that modern obstetrics has to face. The ambiguity in the diagnosis of dystocia is given by a not univocal definition of onset of labour. Indeed, active phase of labour start from 4 cm of dilatation for NICE (3), from 6 cm of dilatation for ACOG (1) and 5 cm of dilatation for WHO (4). This ambiguity leads operators to a greater inclination to diagnose dystocia in a more subjective way than other indications for caesarean sections, to over-diagnose it and, consequently, to treat it in an inappropriate manner. Therefore, a significant concern is due to the fact that many women with a normal progression of labour would undergo unnecessary caesarean sections, just because there is no consensus on the classification and treatment of dystocia. In order to reduce primary caesarean sections in nulliparous at term many authors proposed different strategies.

We need to drastically decrease the subjectivity in diagnosing dystocia in labour (5). It is also necessary to modify the approach in the management of dystocia in labour with the objective of making a diagnosis before considering any therapeutical strategy, in order to reduce iatrogenic interventions

(6). A considerable amount of epidemiological data confirm that iatrogeny in labour is, generally high, and more than half of women giving birth is subjected to oxytocin infusion in labour (7).

From the evolutionary point of view the need to accelerate more than half of the births is unlikely to be real, and it can't be a biological need (8, 9).

The failure to recognize the many different conformations of maternal pelvis, the lack of knowledge of obstetric semeiotics, the widespread practice to neglect foetal position diagnosis (10) from the beginning of labour but, above all, the maintenance of alert and action lines in partograph, an instrument whose utility has not been confirmed by literature (11), led to an improper use of oxytocic perfusion. In a Cochrane review, Bugg demonstrated how the use of oxytocin leads to a modest reduction in the duration of labour, but does not change caesarean section incidence (12). In the ISTAT report "Pregnancy, childbirth and breastfeeding" in 2013, 72.7% of the women interviewed reported they have been subjected to at least one of the following procedures: AROM (32%), episiotomy (34.7%), continuous foetal cardiac monitoring (45.2%), Kristeller manoeuvre (22.3%), oxytocin administration (22.3%), while a small proportion of women, 14.2%, stated they did not know whether forceps or ventouse were used (4.3%) (13).

We should consider how these interventions carried out during labour, especially unscheduled caesarean sections and oxytocin administration would interfere on the mother / child dyad well-being and their consequences on future fertility.

It has, in fact, been demonstrated that the perception of stress secondary to an urgent caesarean section is associated with a feeling of anxiety about future pregnancies (14).

Our attention must be focused on midwives and obstetricians, since a correct management of interventions is strikingly important in maintaining the experience of labour and birth as normal as possible (4, 15).

We want to investigate how much operators are aware of the diagnosis of dystocia in labour and

which management strategies they put in place, in order to understand if their culture can influence their clinical decisions.

For “Awareness of dystocia diagnosis” we mean that operators are able to clearly distinguish between clinical decisions based on subjective diagnostic criteria (influenced by culture) and those based on objective criteria, so that they can provide the right etiologic therapies and avoid unnecessary and dangerous accelerating interventions during labour.

Last but not least, we want to consider how we should manage a prolonged labour in order to make more appropriate and scientific evidence-oriented clinical choices.

MATERIALS AND METHODS

To achieve the objectives set, we chose to conduct a survey among health professionals.

For practical reasons, and because one of our survey aims is discerning whether further research on this subject could be useful, we chose to use a voluntary sample of convenience. The survey was carried out on midwives contacted both personally and via social networks. Moreover, in order to increase participation, we asked for collaboration of different scientific associations, submitting the investigation to their members.

After analysing scientific literature on the scope of the investigation, we decided to use *ad hoc* questionnaire as a data collection tool.

The questionnaire was made up of three sections: socio-demographic information, a clinical case with decision questions and a final questionnaire. 22 questions with multiple answers were edited, with requirement to answer, along with two open-ended questions with no obligation to answer. A presentation letter regarding aim and methods of the research was attached to the questionnaire, in order to motivate participants to answer accurately; it was also specified that the questionnaire was anonymous, and participants could manifest or not their consent.

To reduce the time required to collect data, we created a questionnaire using online survey software (Google Modules) which allowed us to spread the questionnaire, to collect data and process them quickly.

Using this computer system administration, participants were able to easily complete the questionnaire on their electronic devices (smartphone, tablets).

A database was then created to collect all the answers from the questionnaires received using a computerized Excel program.

A descriptive analysis and a graphical representation were carried out to summarize the data.

The graphs used to illustrate the data are pie charts and bar charts.

RESULTS

The survey was carried out from 31st January 2018 to 07th February 2018, and 300 professionals chose to participate voluntarily.

In analysing data we decided to exclude the 11 professionals with a medical degree, given their small number (3.7%), not representative of the category. We therefore analysed data coming from questionnaires filled out by the midwives, in order to focus the investigation on management of dystocia by a sample of Italian midwives. The total sample that we referred to for the analysis is made up of 289 midwives.

All participants gave their consent to take part in the research.

Socio-demographic information

Socio-demographic information show that the most represented age in the sample of midwives is less than 30 in 70.2% of cases, 27% is between 30 and 50, while only 2.8% is over 50 years. 98.3% of the sample population is female. 48.8%, the largest share has 5 years of work experience, 24.2% have never worked, but experienced internships or voluntary attendance, 13.8% have between 5 and 10 years of professional experience, 10% have between 10 and 20 years, and 3.1% have more than 20 years of service.

Clinical case with multiple-choice answers

With regard to the second part of the questionnaire, the results will be presented by citing the proposed clinical case and graphically reporting the responses of the sample population to decision-making questions (**figures 1, 2, 3, 4, 5**).

Retrospectively, 14.7% of the midwives would make different clinical choices.

Regarding the clinical case, 96.9% of the sample would not resolve dystocia with a caesarean section, in contrast to 3.1% that would perform it.

M.B., 29 years old, Caucasian, G1 P0, at 40 + 3 weeks of gestation, uneventful pregnancy, and normal foetal growth. M.B. had contractions every 10 minutes throughout the night, at 8:00 am contractions were every 5 minutes, so she went to the hospital. At 8:30 am she had an obstetric examination: cervix was central, soft, shortened 80%, 4 cm dilated, intact membranes, baby was cephalic with presented part level -3. No information regarding position of foetal head were given. Frequency of contractions was 2 every 10 min. Foetal heart rate was regular. The woman was admitted into hospital.

Which clinical decision do you think is most appropriate now?

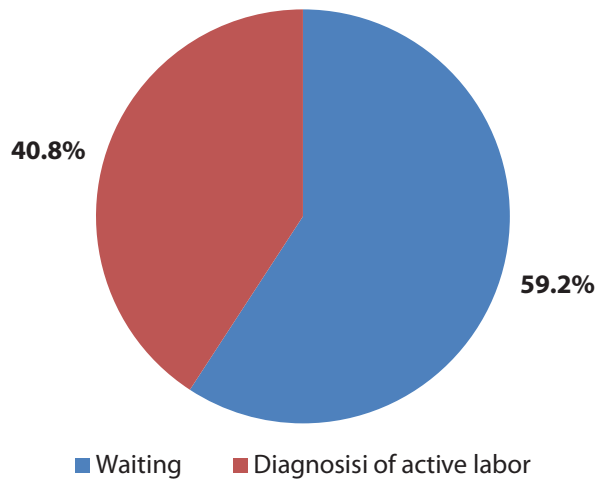


Figure 1. Description of clinical case. First question.

At obstetric triage prodromal labour was diagnosed and the woman was taken to the ward. At 10 am, because of intensification of contractions and pain, an obstetric examination was repeated: uterine cervix was central, fully effaced, 6 cm dilation, presented part -3, intact membranes, frequency of contractions 3 every 10 min. Foetal heart rate was regular. Diagnosis of active labour was made, and the woman was taken to delivery room.

What management would you adopt now?

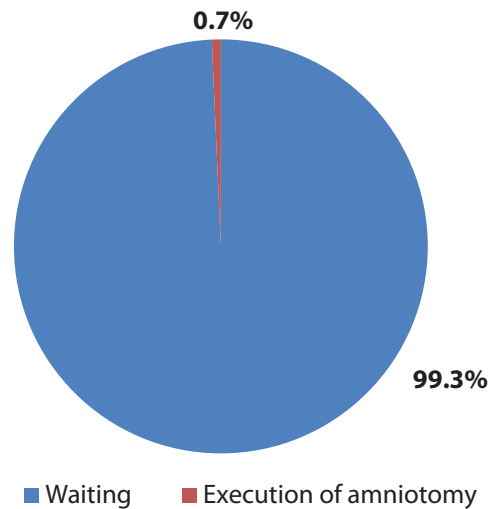


Figure 2. Description of clinical case. Second question.

M.B. reported increased lumbosacral pain so the midwife proposed immersion in the birth pool for an hour, with benefit. Afterwards M.B. got a back massage by her husband. At 2 pm M.B. was examined by the midwife of the afternoon shift. The result was unchanged, frequency of contractions 2 every 10 minutes. Foetal heart rate was still normal on CTG.

What care choice would you make now?

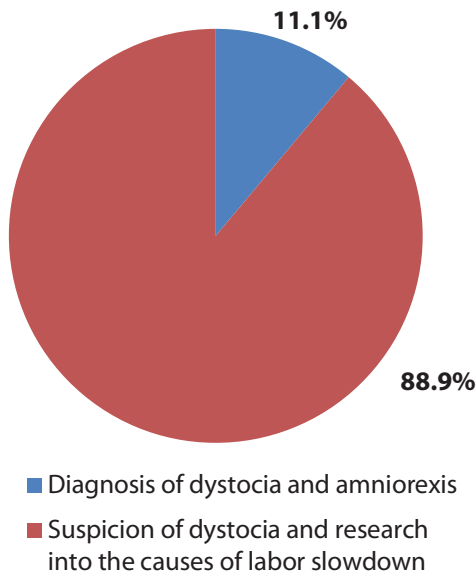


Figure 3. Description of clinical case. Third question.

The midwife reported this examination to the doctor and together they decided to better investigate foetal head position, not reported previously. Foetal back was posterior at palpation, maximum intensity of foetal heartbeat was picked up in the left lateral quadrant, presented part was mobile and not engaged. At vaginal examination, a slightly deflected, left posterior occipital position was diagnosed, which was also confirmed by ultrasound. The midwife encouraged the woman to move and to alternate different positions in order to favour flexion and rotation of the foetal head. She also suggested back massage and sterile water injection to reduce the pain. At 4 pm, vaginal examination was carried out: dilation was 7 cm, foetal head position was still occiput posterior, but now well flexed, level -3, frequency of contractions 3 in 10 min. Regular foetal heartbeat.

What choice would you make now?

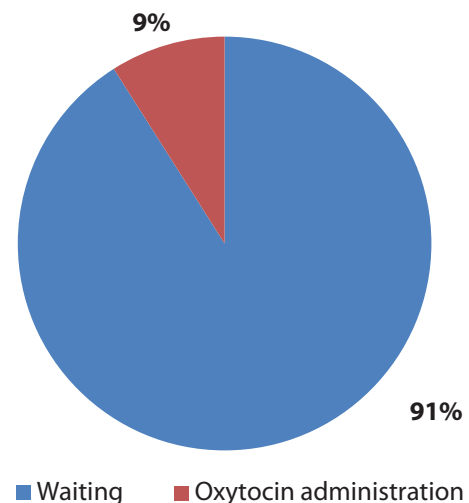
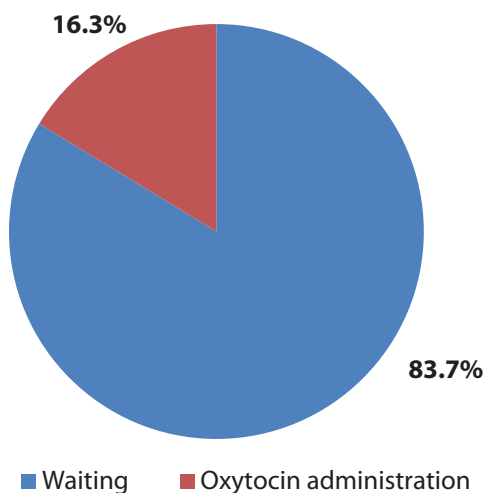


Figure 4. Description of clinical case. Fourth question.

Considering labour progression, the midwife and the obstetrician decided to adopt a conservative management without intervening. Lumbosacral pain increased and M.B. starting feeling urge to push. The midwife suggested again immersion in the birth pool and afterwards the urge to push decreased. At 6 pm vaginal examination was carried out: cervix was 8 cm dilated, presenting part -2 in left posterior occipital position. At that point, exhausted after several hours in labour and excruciating back pain, M.B. requested an epidural. After the first bolus M.B. felt immediate pain relief, positioning herself alternatively on her left side and all-fours. At 9 pm she had spontaneous rupture of membranes: amniotic fluid was clear and foetal heartbeat regular on CTG. New staff took on patient's management and M.B. was examined again: dilatation was now complete, foetal head in left transverse occipital position, level -1. Frequency of contractions was 2 every 10 min.

How would it proceed now?



Operators respected for an hour and a half the physiological period of pause in contractions. Active second stage of labour began at 10.30 pm and at 00.30 there was spontaneous birth of a female newborn, Apgar 9-10 at first and fifth minute respectively, blood loss 400 ml.

Figure 5. Description of clinical case. Fifth question.

Final part of the questionnaire

Moving on to the final part of the questionnaire, it appears that almost all operators investigated (96.5%) reflect upon cases of dystocia faced during their professional experience; 69.9% would do it by themselves, 61.2% discussing cases with other operators and only 12.8% with structured clinical audits.

Table I. Answers at question number 3 of final part of the questionnaire.

| What are the sources of your knowledges that you use in the daily clinical practice? | | |
|--|-----|------|
| Answers | N | % |
| National and international guidelines | 251 | 86.8 |
| University knowledge | 206 | 71.3 |
| Scientific papers | 86 | 29.7 |
| Opinion of experts | 75 | 25.9 |

The knowledge on which participants base their clinical practice is based on national and international guidelines in 86.8% of cases, on university knowledge in 71.3%, on scientific papers in 29.7% and on opinion of experts in 25.9% of cases (table I).

76.8% of midwives investigated agree there is currently no unambiguous definition of labour dystocia, 12.1% would define dystocia as a lack of cervical dilation for more than two hours and finally, 11.1% think that dystocia occurs when labour proceeds at less than 1.2 cm/hour in nulliparous women.

The sample population was then asked which therapeutic treatments they most frequently use in clinical practice when they deal with a dystocic labour (table II).

The three most frequent choices are maternal movement (71.6%), change of maternal posture (81%) and emotional support (40.5%).

To the question: "How would you define latent phase of labour?" 80.3% of participants answered that it begins at complete dilation and ends when the patient feels the need to push, 17% defined this phase as the time between the appearance of regular contractions and acceleration of cervical dilation; finally, 2.8% stated that latent phase is the interval between beginning of labour and complete dilation.

The different protocols of oxytocin administration in labour were then investigated. 58.8% of midwives believe that timing of oxytocin administration depends on foetal position and on clinical context, 28% think it is better to start oxytocin with ruptured membranes, 11.4% with intact membranes and, for 1.7%, the method is irrelevant.

Table II. Answers at question number 5 of final part of the questionnaire.

| Which therapeutic treatments most frequently do you use in clinical practice when do you deal with a dystocic labour? | | |
|---|-----|------|
| Answers | N | % |
| Oxytocin | 71 | 24.6 |
| AROM | 85 | 29.4 |
| Analgesia | 32 | 11.1 |
| Maternal movement | 207 | 71.6 |
| Change of maternal posture | 234 | 81 |
| Emotional support | 117 | 40.5 |
| Massage | 51 | 17.6 |
| Use of hot water | 89 | 30.8 |
| Acupuncture | 0 | 0 |
| Supporting by Hand | 10 | 3.5 |
| Chromotherapy | 0 | 0 |

Participants were also asked whether or not they follow specific protocols for the administration of oxytocin, whether or not they use an infusion pump to administer it and how they monitor foetal well-being (**table III**). 87.8% report to use oxytocin in labour with a specific protocol, while 8.3% without. 65.4% of midwives investigated always administer oxytocin using an infusion pump, 12.8% only in certain circumstances, and 7.9% never use an infusion pump.

Last but not least, during oxytocin perfusion, foetal heart rate is monitored through continuous cardiotocography in 96.5% of cases, and with intermittent auscultation in 0.7% of cases.

The propensity of midwives towards spontaneous delivery was then investigated, asking them which percentage of primiparous women at term in spontaneous labour with foetal cephalic presentation could deliver vaginally, without resorting to caesarean section. 76.8% of the sample answered that every woman can deliver vaginally, 19% think that 50% of this category of women needs a caesarean section to be delivered and 2.4%, 1% and 0.7% respectively believes that only 3-7%, 13-20% and 20-30% can give birth without caesarean section.

Finally, investigating the causes of inadequate diagnosis and management of prolonged labour and the consequent increase in iatrogenic interventions emerged that the most important causal factors among those proposed were the culture of medicalization of birth (77.5%), errors in the diagnosis of active labour (74%) and inadequate knowledge and/or failure to update (53.3%).

DISCUSSION

The majority of the examined sample is women, very young, 70.2% under 30, and with little work

experience (48.8% worked less than 5 years and 24.2% had never worked at all and had only experienced post-graduate training and/or voluntary attendance).

Despite the short period of administration of the questionnaire, detecting an important participation by midwives, especially young ones, who represent the future of this profession, is expression of interest in the issue we address.

Moving on to non-personal data, let us take a look at how midwives managed the clinical case we proposed.

The most notable points we wanted to investigate were diagnosis of active labour, diagnosis of dystocia and interventions performed during labour.

With regard to diagnosis of active labour, more than half of midwives (59.2%) would not have made diagnosis of active labour before 4 cm of dilation and in the absence of valid contractile activity (**figure 1**). This reflects the current scientific evidence that shifts the beginning of active labour, hence a phase of acceleration of cervical dilation rate, from 4 to 6 cm. Zhang *et al.* in fact revolutionized the diagnosis of active labour and the way to manage labour duration, leading to new definitions of latent phase and active phase (16).

40.8% of midwives who, instead of waiting, would make diagnosis of active labour at 4 cm dilation (**figure 1**), probably refer to the old definitions dictated by Friedman, resumed, and partly modified, by authoritative scientific institutes such as NICE and WHO (3, 17).

Regarding the timing when dystocia is diagnosed, it is interesting to note that in the presence of an unchanged finding 4 hours apart (6 cm dilation, PP-3 level, regular contractions) 88.9% of operators would opt for a wait-and-see management and investigate causes of labour slowdown, while only 11.1% of midwives would diagnose dystocia at this point and proceed with rupture of the membranes (**figure 3**).

Again, most of the interviewees were oriented to act according to recent scientific evidence, which considers the progression pattern of labour unique for every single woman instead of trying to standardize it (18). There is a clear need to investigate causes when a slowdown in labour progression is suspected and there is a need for operators to adopt other methods to correct anomalies, thus limiting interventions such as rupture of membranes, oxytocin administration and caesarean sections, that can be associated to negative consequences.

Table III. Answers at question number 8 of final part of the questionnaire.

| How do you use Oxytocin for labour augmentation? | | |
|--|-----|------|
| Answers | N | % |
| We follow a specific protocols | 245 | 87.8 |
| We have not a specific protocols | 24 | 8.3 |
| We use always an infusion pump | 189 | 65.4 |
| We use sometimes an infusion pump | 37 | 12.8 |
| We never use an infusion pump | 23 | 7.9 |
| We use CTG to BCF monitoring | 279 | 96.5 |
| We use intermittent auscultation to BCF monitoring | 2 | 0.7 |

In this perspective, the hyperbolic curve deriving from cervical dilation /time ratio becomes a screening tool and no longer a diagnostic tool to decide when to intervene with medical procedures (6, 19).

11.1% of midwives who would intervene with artificial rupture of membranes at the diagnosis of dystocia, probably, as indicated by a comment on the questionnaire, would do it in accordance with NICE indications, that support rupturing the membranes at the time of delay in cervical dilation after 4 hours of unchanged finding (**figure 3**).

We believe that at the base of this management there is not only the intention to follow the guidelines but also the belief, of cultural derivation, that the genesis of dystocia is a hypo-valid contractile activity and that accelerating labour always brings advantages.

In conclusion, with regard to the management of the clinical case proposed and in contrast to what we expected, it emerged that our midwives would prefer a wait-and-see approach and respect physiology. With regard to a prolonged labour, due to a foetal head malposition, midwives declare they would rather wait than perform rupture of membranes or administer oxytocin. Finally, consistently with the wait-and-see attitude adopted during the clinical case, only 3.1% of midwives decided to perform a caesarean section. The fact that among objectives for care provision in labour there is a strong determination to maintain labour as normal as possible, reducing disturbing interventions, is a sign that a cultural change can and must be implemented.

Interviewees were then asked to reconsider the choices made regarding the clinical case: 14.9% of them, looking back, stated they would change the decisions taken. Many would change the time they diagnosed active labour, others would avoid oxytocin administration and rupture of membranes, others would try more to look for underlying causes of dystocia, rather than labelling right away that labour as abnormal and deciding to intervene.

One reply in particular highlighted an aspect that characterizes many Italian hospitals.

It is very interesting to understand how the work context, the workload, the education everyone received, and the work experience can influence operators who often do not operate as they would like, but on the basis of what is dictated from the culture that surrounds them (20).

Moving on to the final part of the questionnaire, it is very interesting that there is debate on cases

of dystocia faced during professional experience (96.5% of midwives), but that the discussion is rarely coordinated in a structured context (audit is performed only in 12.8% of cases). Clinical audits compared to individual revision of clinical cases alone or to unstructured meetings between operators, are certainly more productive. Audits represent indeed tools able to identify problems that occurred and to relate them to the best scientific evidence, but also an opportunity for updating, for professional growth and to make new proposals in order to improve clinical practice and not to repeat the same mistakes.

It is very relevant that, probably due to the recent Gelli-Bianco law (Law 24/2017) on medical responsibility and liability, in 86.8% of the sample the sources of knowledge are based on national and international guidelines (**table I**).

But what we ask is: "Can we understand clinical reality just reading guidelines?". We can certainly learn something, but a deep analysis is needed starting from the context in which we operate, personalizing assistance and relating with all operators in a "team work" perspective.

We were pleased to find that, to the question "What is dystocia?" 76.8% of operators answered that there is no single definition, a sign that this knowledge has spread among Italian midwives. Dystocia is a complex syndrome, rather than a single disease attributable merely to a single cause (**figure 6**) (6). Consistent with the decision to adopt a wait-and-see management, in case of prolonged labour, operators interviewed used mainly these three interventions: change of maternal posture, movement and emotional support rather than intervening au-

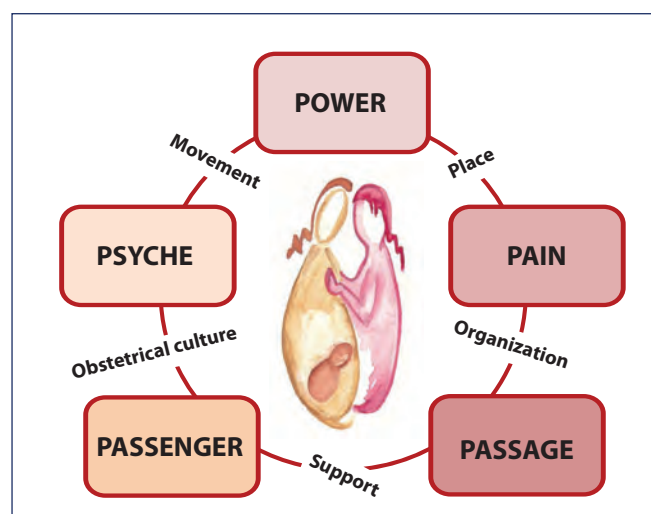


Figure 6. The complexity of childbirth.

tomatically with rupture of membranes and oxytocin administration (**table II**).

The systematic review by Lawrence *et al.* (2013) highlights a clear benefit from maternal movement and especially from vertical posture, in terms of reduction of first stage of labour, of epidural request and of caesarean sections performed for labour dystocia. At the same time maternal and neonatal well-being is greater (21).

In addition to physical well-being, it is essential that also psychological and emotional well-being of the woman are respected, since a neuro-endocrine system imbalance is one of the causes of prolonged labour (9).

It is very interesting to observe how almost all of midwives (80.7%) gave a wrong definition of latent phase, mostly confusing it with transition phase. Ignoring latent phase or considering it part of active labour could contribute to a wrong diagnosis of dystocia and consequently lead to early and unnecessary medical interventions. Knowing how to recognize latent phase of the first stage and knowing how to distinguish it from the active phase leads to a decrease in interventions during labour (22).

In addition to confusion over definition of latent phase, it is interesting to highlight how 58.8% of the sample evaluate and take into account foetal position before artificial rupture of membrane or oxytocin administration. In literature there is no clear indication about which of the two interventions should precede the other. Different studies report that early or late oxytocin administration after rupture of membranes reduce labour duration, thus favouring vaginal delivery (23). Other studies established there is no real difference in clinical outcomes between oxytocin administration with ruptured or intact membranes (24). In a 2013 RTC Tan *et al.* concluded that the choice between the two options should take into account local resources and the woman preference (25).

Regarding oxytocin administration, the majority of midwives use it following specific protocols (87.8%), always giving it through an infusion pump (65.4%) and monitoring foetal heart rate and contractile activity continuously (96.5%) (**table III**). What is surprising is that despite oxytocin is a potentially dangerous drug, there are still hospitals where the infusion pump is not used at all (7.9% of the sample) or only in certain circumstances (12.8%) and without specific protocols (8.3%) (**table III**). In its document "Managing Complications in Pregnancy

and Childbirth: A guide for midwives and doctors", in the section dedicated to augmentation of labour, WHO reports how oxytocin should be correctly administered, therefore dosage, infusion rate and the maximum frequency and intensity of contractions achieved. It is also indicated that during oxytocin infusion foetal heart rate should be monitored in continuous and one-to-one care should be provided, since it's greater the likelihood of developing complications such as uterine hyper-stimulation or pathological CTG (26).

The question about which percentage of women (primiparous in spontaneous labour, at term with cephalic foetus) can give birth spontaneously is put deliberately in a incorrect manner, but helps to explore the propensity of midwives to be positive or not. In fact, 76.8% replied that all women in this category can give birth vaginally, which is obviously impossible, but this answer shows that the majority of midwives have a positive attitude to the possibility that women can give birth spontaneously.

At the end of our study, we investigated what midwives think could be the causes of inadequate diagnosis and management of prolonged labour, with the consequent increase in iatrogenic interventions. The most significant reasons detected were a culture of medicalisation of birth event (77.5%), errors in diagnosis of active stage of labour (74%), willingness to adapt to the "habits" of the workplace (46.7%) and inadequate knowledge and/or failure to update (53.3%). Data collected by this survey let us understand which are the most relevant problems to be addressed and in which direction to work to improve assistance. It is certainly clear the need for a better training for doctors and midwives, and a greater dissemination of knowledge of obstetric semeiotics and of maternal postures in labour. It is therefore important to respect times of different stages of labour. Furthermore, dissemination of shared protocols on the criteria to diagnose active stage of labour and so then, possible dystocia, together with respect for women and their own individual times in labour, will certainly improve maternal and neonatal outcomes.

One of the limits of our research is that the sampling was carried out with non-probabilistic methods, therefore not offering all the population the same possibility to become part of the research, so the sample is not representative of the population itself. Another limit that can be taken into account is the distortion caused by incomplete answers, even if

they represent a very small number. In fact, incomplete answers concern only one question of the questionnaire and involve 16.3% of total sample. Lastly, we consider a limit of our research the fact that the sample is composed only of midwives. In fact, analysing our results, we could only assess the point of view of the category of midwives who, however, are not the only operators to interface with women and to manage their labour.

CONCLUSIONS

In conclusion, our investigation performed on a sample of Italian midwives, revealed that labour dystocia is certainly a topic that is discussed and that arouses interest. We observed that the majority of midwives interviewed have a propensity to respect physiology of birth.

There are also gaps to be filled in knowledge and barriers to be overcome concerning cultural aspects, but the fact that there is a strong will to maintain labour as normal as possible, limiting any disturbing intervention, is already a signal that cultural change can and must be implemented.

Strategies that can be put in place to deal with the problems encountered are:

- investing more in training midwives and doctors;
- organizing structured meetings such as clinical audits in order to collegially discuss clinical cases;

- spreading scientific evidence on dystocia and disseminating a new concept of dystocia;
- defining it as a syndrome and not just as a slow progression of labour.

It would be interesting, in the future, to extend this survey to a greater number of obstetricians and midwives working together as a team to better define the culture of labour ward operators and to help them to do appropriate clinical choices in order to reduce as much as possible iatrogenic interventions in labour and guarantee women a positive birth experience (4).

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CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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Supra- versus Infra-pannicular skin incision in cesarean section: surgical techniques

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Cesarean section; surgery; panniculus; skin incision; delivery.

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To the Editor,

Cesarean section is rising now with an estimated prevalence in the UK about 25% of deliveries and in Egypt, a developing country, it is around 55% and maybe more. Obesity and overweight patients with BMI over 30 are rising now due to poor lifestyle and unhealthy diet. Obesity prevalence in the UK is around 30% and it is rising. So, we commonly encounter obese patients scheduled for cesarean section whether as elective or emergency cases. Cesarean section is high in obese women due to poor progress of labor, large baby, abnormal position and presentation.

Regarding the cesarean section, the available evidence and the number of questions still debated about the best surgical approach, there is no standard practice for cesarean section technique. As the following examples shown here, there is no evidence or effect for peritoneal closure to justify the additional time and use of suture material along with no effect on fever or healing. Also, no difference between a sharp or blunt extension of the uterine incision apart from the associated lower blood loss and lower need for blood transfusion in the

blunt type. Catgut closure *versus* polygalactin was associated with a lower blood transfusion and complications in the catgut group. Single-layer closure can lower blood loss with respect to a double-layer one. Pelvic pain, adhesions, infertility, deep dyspareunia, hysterectomy, and outcomes of subsequent pregnancies following blunt *versus* sharp entry, exteriorization of the uterus *versus* intra-abdominal repair, single *versus* double-layer closure of the uterus, closure *versus* non-closure of the peritoneum and chromic catgut *versus* polygalactin were evaluated and there was no difference. The type of the technique used to perform the cesarean section does not influence significantly the outcome. Double-layer closure is associated with a thicker 35-38 weeks third-trimester lower uterine segment thickness > 2.0 mm but no difference in the uterine dehiscence or uterine rupture. The debate is still open regarding residual myometrial thickness alone could be the main important risk factor or not. Monofilament sutures may improve uterine scar healing without increasing costs, operation time by a larger thickness of the multifilament suture (1). Obstetricians performing cesarean section should evaluate for risk factors for complications as bladder

injury, bowel injury, bleeding, *etc.*, and promptly recognize and repair the lesion. Identification and immediate repair are of paramount importance reducing the risk of further surgical procedures and complications. Previous cesarean obesity and peritoneal adhesions are the main risk factors for complications (2). Regarding obesity, obese patients have high body fat in the abdomen around the viscera and in the pelvis, so obstruction during delivery or soft tissue dystocia is common in opposition to the old beliefs that fat tissue weight on the pelvis can widen the pelvic dimension by pressure and favors easy delivery. Excess fatty tissue can hinder delivery by soft tissue obstruction.

Cesarean section in obese women is technically difficult due to deep pelvis, deep suturing needed, long instruments needed, an engorged uterus encountered, varicose veins especially in the broad ligament with high risk for injury and hematoma formation, even on gentle manipulation without traumatic injury and infection is also common.

A skin infection can occur in 10% after the cesarean section and is more in obese women. The subpannicular fold is so wet from sweat and so is rich in bacteria and also poorly related hygiene increases susceptibility to infection.

Regarding surgical technique in obese women, the skin incision can be supra or infra-pannicular one, we are going to discuss them in detail and their role along with the techniques to avoid skin infection in these women.

The supra-pannicular skin incision is usually performed above the panniculus through the large subcutaneous fat as a high transverse or vertical incision below the umbilicus after pulling the panniculus down. The limit of the vertical incision is the felt symphysis pubis through the panniculus and the transverse incision is wider due to difficult fetal extraction and delivery from the deep abdominal and uterine cavity.

The infra-pannicular skin incision is the transverse one below the panniculus away from the reflection of the panniculus at the usual Pfannenstiel incision near symphysis pubis but away from the sensitive pubic hair area. The fatty layer here is thinner and a smaller narrower incision is used, and the pelvic cavity is reached more easily and quickly.

We may need to elevate the panniculus by an adhesive non-allergic tape to the top or sides of the operating table. This limits assistant surgeon fatigue while raising the panniculus during the potentially prolonged surgery-suprapannus transverse skin

incision is associated with more bleeding on subcutaneous dissection, infection, more tissue dissection, need for drain seroma formation, subtheath hematoma and dehiscence related to infection and poor cosmetically. Regarding the vertical one it is associated with more pain, difficult breathing, difficult early ampulation and not good cosmetically. It can be used with abdominal masses that difficult to be approached from the pelvis (figures 1, 2).

The infra-pannus skin incision is associated with a lower incidence of skin infection due to the thin fatty layer, rapid entry, less vascular area, away of the moist reflection, interrupted skin suture, less bleeding and hematoma formation and shorter duration of surgery. Daily cleaning after exposure after 24 hrs and keeping the area dry from sweat and water especially with perineal hygiene is very important. Antibiotic prophylaxis is needed for 7 days especially in diabetics with poor control.

If the panniculus is redundant down after delivery covering the incision site, we proceed with interrupted sutures. If after delivery the panniculus is above the incision site and will not go down on motion then subcuticular sutures can be used and management is as non-obese patients.

Regarding techniques for obese patients' surgical technique, we incise the subcutaneous tissue sharply at the midline then bluntly with fingers laterally exposing the rectus sheath. Avoid dissecting the subcutaneous layer below the skin edges to

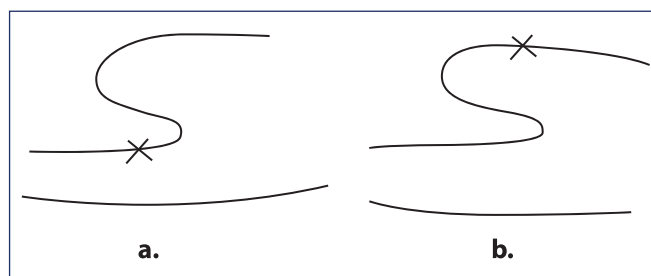


Figure 1. Suprapannicular versus infrapannicular skin incision.

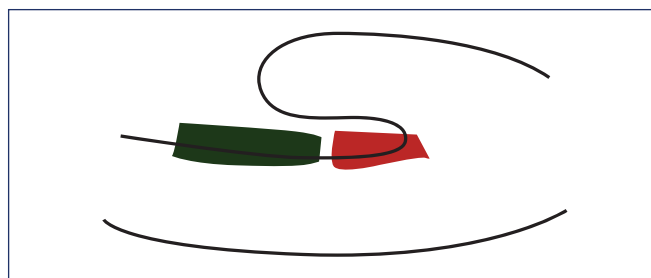


Figure 2. Avoid the red area and incise in green area that can be uncovered by a pulling up dressing or at the exposed area below.

avoid seroma formation. Incise the sheath sharply at midline then bluntly till the skin edges at the angles and not beyond to avoid puckering of skin on suturing the sheath or missing the beginning of sheath on suturing leaving a defect. Make the skin incision wide enough to avoid dissecting the rectus sheath from muscle and provide more space for the fetal delivery. This will prevent sub-rectal sheath hematoma that can present early as hematoma or hypovolemic shock as hematoma can extend down to the vulva that can be seen easily there as thinner skin than the thicker abdominal one that can accommodate huge amount before being visualized or can appear commonly as skin discoloration and infection after two weeks-incise the uterus high up at the level of the skin incision not low down as difficult suturing later as difficult deep suturing in the deep pelvis by large skin thickness. It is better to take care of the engorged large varicose veins of the broad ligament. If injured, ligate the ligament down below them to avoid extension of hematoma retroperitoneal leaving the ovary with blood supply from the uterus even if it shows black spots of venous congestion, it can regain vitality once collaterals and uterine supply increase over time as long as no ovarian ligament ligated. Also, this can be managed by ligating part of the broad ligament, if not largely affected and the ovarian vessels can be preserved. It is better to proceed with uterine exteriorization to suture the uterus easily instead of doing that in the deep pelvis with short instruments. The uterus is commonly becoming congested or engorged on exteriorization for a long period, so reduction may be difficult or traumatic and it is better to be done gently with a towel and a retractor along with ensuring sub sheath hemostasis. No drains should be left in the subcutaneous tissue to avoid infection but hemostasis is important and closure

of the dead space, especially below the skin angles. DVT prophylaxis is essential. Long-standing diabetes is commonly associated with vasculitis and fibrosis of the tissues so tissues are usually tough especially the subcutaneous tissue on incision and can tear easily as non-elastic and dissect more difficulty with a scalpel as fibrosis (3, 4).

We need to incorporate these technical tips in the learning of the residents and physicians. The European training programs in ob-gyn still vary significantly. A new European training curriculum encompassing all the competencies required of a newly trained specialist is an essential step for harmonization of training and standardization of care (5).

The professional competence scale consisted of eight dimensions with a total of 51 items was proposed that can be used for training and evaluation of physicians these dimensions are essential medical knowledge public health or social science essential clinical skills advanced clinical skills communication skills advanced study skills critical thinking and adaptation and professionalism (6).

ETHICS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from the patient included in the study.

CONFLICT OF INTERESTS

The author declares that he has no conflict of interests.

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