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# Italian Journal of Gynæcology & Obstetrics

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## Italian Society of Gynaecology and Obstetrics (SIGO): Consensus paper on induction of labor with oral administration of Misoprostol

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### INTRODUCTION

Induction of labor (IOL) is a medical procedure used to initiate labor and conclude the pregnancy. Its primary aim is to stimulate and obtain an active birth labor. A procedure is appropriate (inappropriate) if its expected benefit is sufficiently higher (lower) than its expected cost (1-4). For this reason, IOL should only be taken into consideration when it is clearly beneficial to either mother and/or fetus and thus reduces the exposure to risks and increases maternal and/or fetal wellbeing. The risks related to IOL must be lower compared to the risks that the continuation of the pregnancy may lead to (5).

The incidence of IOL in Europe varies between 6.8% and 33% (6). This percentage varies greatly in countries worldwide and has shown a general and progressive growth in recent years. As regards the situation in Italy, statistics show that in some realities the percentage of induced labor exceeds one in every four births, with an average of 23.2% in 2017 (7).

General consensus for IOL can be found in at least three specific circumstances:

- a. term pregnancy and prevention of post-term pregnancy;
- b. pre-labor rupture of amniotic membranes (PROM);
- c. fetal death.

Though not clearly beneficial to mother and/or fetus, IOL is being used in various other circumstances, such as:

1. hypertension (gestational, preeclampsia);
2. fetal growth restriction (IUGR/SGA);
3. cholestasis;
4. diabetes.

Other, more controversial circumstances, include:

1. request of the pregnant woman;
2. logistic and/or social purposes;
3. oligoamnios;
4. polyhydramnios;
5. fetal conditions that require a planned birth for any potential surgery.

The medical team must discuss all circumstances with the pregnant woman.

An induced labor generally tends to be longer compared to a spontaneous labor; this can impact the pregnant woman significantly from a psychological point of view (8, 9). The factors that impact the most on the outcome of an IOL are the patient's compliance to the procedure and continuity of care (10). The two main approaches to IOL are either the mechanical technique or the use of pharmacological agents, this second being the most common with the use of prostaglandins. Prostaglandins can be administered in various forms. The optimal mode and dose of administration are currently the object of various studies and scientific researches (11).

The interval of time between the beginning of an induction and the onset of active labor is one of the key factors that influences the patient's satisfaction. In fact, a study (12) comparing different variables between 450 women who labored spontaneously and 450 women who were induced demonstrates that:

1. 34.7% of women with an induced labor aren't satisfied with the information received;
2. 27.2% expect to give birth within 12 hours from the first medical procedure used for IOL;
3. the first thing 40% of the patients would change, if they were to have another induction, is the speed of the induction;
4. women that receive an IOL are generally less satisfied of their birth experience compared to women who have a spontaneous labor (70.4% vs 79.5%).

## MISOPROSTOL

Misoprostol is a synthetic analogue of PGE1, initially used to prevent damages to the gastric mucosa and to prevent and treat gastric and duodenal ulcers. Misoprostol is economical, stable at room temperature and available in more than 80 countries worldwide, making it a fundamental drug in areas with limited resources (13). The World Health Organization (WHO) has included Misoprostol in the list of essential drugs due to its properties and its optimal profile in terms of costs and benefits, thus resulting in an extremely efficient, appropriate and economic drug (14). Misoprostol explicates its action on the cervix by stimulating contractions and facilitating cervical ripening and dilatation (15). An extended bibliography supports the evidence and recommendations that confirm Misoprostol to be superior and more efficient compared to drugs containing PGE2 (16-23). Wanting to be more specific, compared to the other classes

of prostaglandins, Misoprostol shows greater efficiency and results in:

1. a shorter interval of time between induction and birth;
2. greater probability of a vaginal birth within 24h from induction;
3. greater safety in case of PROM;
4. lower risk of caesarean.

The recommended dose of Misoprostol is 25 mcg per os every 2 hours, for a maximum of 8 doses. It is recommended to dilute 200 mcg of Misoprostol in 200 ml of water and administer 25 ml of the solution every 2 hours. The administration of the drug must be preceded by at least 30 minutes of cardiotocography to ensure the wellbeing of the fetus and frequency of uterine contractions. CTG monitoring must also continue for the first hour following the administration of Misoprostol. It is important to keep in mind Misoprostol has effect after about 10 minutes of administration.

Currently, oral Misoprostol is the only form available for gynaecological use. Scientific literature suggests that oral administration of Misoprostol with low doses is linked to a lower risk of C-section and tachysystole compared to vaginal administration.

### *Misoprostol 25 mcg: "ANGUSTA®"*

In January 2021, the pharmaceutical drug containing 25 mcg Misoprostol "ANGUSTA®" was authorized and released on the Italian pharmaceutical market under the form of tablets. This solution is therefore available at the recommended dose; due to this, no additional preparation is needed before the administration of the medicine to pregnant women. The maximum dose must not exceed 200 mcg in 24 hours (maximum 8 tablets/24h). The posology recommended on the technical data sheet (TDS) is 25 mcg every 2 hours or 50 mcg every 4 hours.

### *Proposed protocol for induction of labor with Misoprostol 25 mcg*

Based on data from Scandinavian studies (24), it is reasonable to propose the administration of one 25 mcg tablet per os every 2 hours, for a total of 6 doses on the first day of induction. In case of failure, this procedure can be repeated on day two, with a maximum of 8 doses. It can eventually be repeated on day three with a maximum of 3 doses. According to results available in scientific literature, around 70%

of women give birth within 48 hours from the beginning of induction. Seeing as studies concerning the duration of treatment for induction and precise posology for Misoprostol are scarce, it is retained fully acceptable for centres to follow different protocols and to autonomously decide when and/or if to eventually interrupt treatment. Compared to the use of high doses (50 mcg), induction with Misoprostol 25 mcg shows a more favourable obstetric outcome and a higher probability of a successful vaginal birth. The actual percentage of an assisted birth with vacuum or forceps following IOL with Misoprostol is yet to be defined, as some studies hypothesise that low doses (25 mcg) of Misoprostol may increase this risk (25). The first dose of Misoprostol must be administered after a 30-minute reactive Non Stress Test (NST) (26). During the first day of induction, the dose may be repeated every 2 hours and up to 6 times for the Scandinavian protocol or up to 8 times according to the drug's TDS. The maximum dose on day 2 of induction is 8 doses (total 200 mcg) for both the Scandinavian protocol and the TDS, while the maximum dose on day 3 is 3 doses (75 mcg).

The uterine response to the drug must always be evaluated manually during and between the administration of the doses. Administration must not be automatic but preceded by the evaluation of any ongoing labor conditions, even in latent form, circumstance which contraindicates a new administration of the drug. It is recommended to monitor fetal wellbeing and uterine activity with a 60-minute NST after the first dose. Further monitoring of fetal wellbeing and uterine activity depends on the protocols used and the experience each medical team. Should the administration of oxytocin be made necessary, it is recommended to wait at least 4 hours from the administration of Misoprostol. Hospitals offering IOL must guarantee the possibility to proceed with an emergency cesarean section. The authors recommend paying special and close attention to multiparous women with three or more previous vaginal births or in case of a twin pregnancy.

### ABSOLUTE CONTRAINDICATIONS

1. Hypersensitivity to Misoprostol or any of the excipients contained in the drug;
2. evolving labor;
3. administration of oxytocic drugs and/or other IOL procedures in the same frame time;
4. presence of uterine scars from previous surgery;
5. uterine malformations that contraindicate vaginal birth;
6. placenta previa;
7. abnormal presentation of the fetus;
8. patients with kidney failure (glomerular filtration rate 15 ml/min/1.73 m<sup>2</sup>).

### RECOMMENDATIONS

1. IOL with Misoprostol 25 mcg should only be offered in circumstances in which scientific evidence clearly points out that the benefits deriving from anticipating labor are greater than the potential risks that can derive from the procedure used for IOL itself.
2. Indications for IOL, aside from those commonly accepted such as term pregnancy, post term pregnancy, PROM and fetal death, must always be discussed and coordinated collectively by medical staff.
3. The procedure intended to be used for IOL should be thoroughly discussed with the pregnant woman prior to its onset. It is important to provide information regarding the various procedures normally brought forward throughout labor, the benefits and potential complications that could arise. The chosen procedure and the average time for labor onset and birth should be clear to the patient.
4. Informed consent must be obtained and signed by the pregnant woman prior to commencing the induction. The possibility that a full cycle of induction with Misoprostol may not be sufficient to stimulate labor and/or that the patient may not respond must be explained. If this were to be the case, the patient must also be informed that other attempts may be made using different approaches; these can either be pharmacological or mechanical. Another scenario is that in which the woman refuses to carry on the induction, opting for a C-section instead; this represents a "refusal of continuation of IOL" and not "failure of IOL".
5. The definition of "failure of IOL" is given by the inability to reach active labor (regular and efficient uterine activity with 2-4 contractions every 10 minutes, a cervix effaced by at least 80% and progressive dilation greater than 4-5 cm) after at least 15 hours of oxytocin and ruptured membranes (spontaneous or whit amniorexi).

- Misoprostol can be considered the first pharmacological therapeutic approach in case of IOL in women that have reached 37<sup>+0</sup> weeks of gestation, have a Bishop score lower than 7 and in absence of any absolute contraindications.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## Multiple human papillomavirus infection and ASCUS-LSIL progression: a review

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### ABSTRACT

Recent literature highlighted the role of Multiple Human Papillomavirus (HPV) infections in the development of CIN2<sup>+</sup>. A better understanding of single-genotype and combined multiple-genotype oncogenic potential has become essential to plan future screening and to evaluate the prospective susceptibility to high risk cervical lesions progression. Analysing the literature data, we evaluated the prevalence and the prognostic significance of multiple HPV infections and their type-specific interactions in women with ASCUS and L-SIL cytology. Multiple HPV infections are detected in about 16.7% of women with ASCUS cytology and in 28.7% of LSIL Pap smear results. In ASCUS patients the rates of severe biopsy proven lesions are 8.6% and 31.6% in women with single and multiple HPV infections respectively. Similarly, in women with LSIL cytology, the likelihood of CIN2<sup>+</sup> is 12.5% in patients with single and 28.4% in those with multiple HPV infection. Despite the well-known oncogenic risk of HPV16 and HPV18, recent studies provide a new insight in the high prevalence of other HR-HPVs and their significant contribution to a large proportion of high-grade cervical lesions in women with ASCUS/LSIL. This review provides further evidence that multiple HR-HPV infection is a significant risk factor for severe cervical lesions in women with ASCUS and LSIL cytology, and highlights that increased oncogenic risk might be strictly associated with peculiar type-specific profiles.

### SOMMARIO

La letteratura recente ha evidenziato il ruolo delle infezioni multiple da papillomavirus umano (HPV) nello sviluppo di CIN2<sup>+</sup>. Una migliore comprensione del potenziale oncogeno del genotipo singolo e dei genotipi multipli è diventata essenziale per pianificare lo screening futuro e per valutare la potenziale suscettibilità della progressione delle lesioni cervicali ad alto rischio. Analizzando i dati della letteratura, abbiamo valutato la prevalenza e il significato prognostico di più infezioni da HPV e le loro interazioni tipo-specifiche nelle donne con citologia ASCUS e L-SIL. Infezioni multiple da HPV vengono rilevate in circa il 16.7% delle donne con citologia ASCUS e nel 28.7% dei risultati del Pap-test LSIL. Nei pazienti ASCUS le percentuali di lesioni gravi comprovate da biopsia sono dell'8.6% e del 31.6% nelle donne con infezioni da HPV singola e multiple, rispettivamente. Allo stesso modo, nelle donne con citologia LSIL, la probabilità di CIN2<sup>+</sup> è del 12.5% nelle pazienti con infezione da HPV singola e del 28.4% in quelle con infezione multipla da HPV. Nonostante il ben noto rischio oncogeno di HPV16 e HPV18, studi recenti forniscono una nuova visione dell'elevata prevalenza di altri HPV HR e del loro contributo significativo a un'ampia percentuale di lesioni cervicali di alto grado nelle donne con ASCUS/LSIL. Questa revisione supporta il razionale che l'infezione multipla da HPV HR è un fattore di rischio significativo per lesioni cervicali gravi nelle donne con citologia ASCUS e LSIL e sottolinea che un aumento del rischio oncogeno potrebbe essere strettamente associato a profili specifici per tipo particolare.

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**Key words**

ASCUS; high-grade CIN; human papillomavirus; LSIL; multiple infection.

## INTRODUCTION

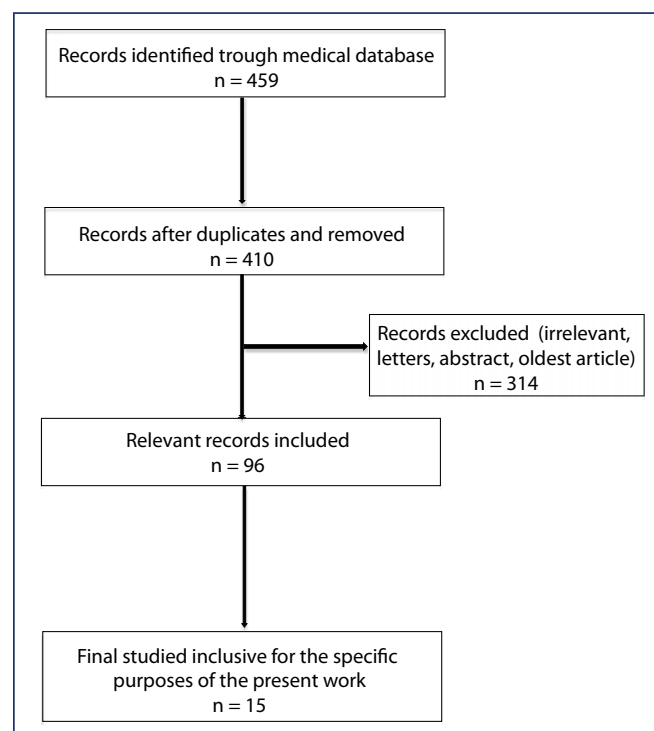
Multiple Human Papillomavirus (HPV) infections in Cervical Intraepithelial Neoplasia (CIN) have gained increasing attention in recent literature. To date, both the biology of inter-genotypic molecular interactions and their clinical implications are yet unclear, even though greater awareness would be necessary for enhanced primary and secondary prevention programs. A large number of surveys (1), point out that immunity towards HPV is predominantly type-specific, with limited cross-action. Hence, a better understanding of single-genotype and combined multiple-genotype oncogenic potential has become essential to plan future vaccination schedules and to evaluate the prospective susceptibility to high risk cervical lesions caused by no-targeted high risk (HR)-HPV genotypes in vaccinated cohorts (2).

Most of the published population data addressing the role of multiple HR-HPV infections on the severity of cervical dysplasia in women with abnormal cytology consider co-infections as a whole rather than highlighting the interactions of single HPV genotypes. Analysing the literature data, we evaluated the prevalence and the prognostic significance of multiple HR-HPV infections and their type-specific interactions in women with Atypical squamous cells of undetermined significance (ASCUS) and Low-grade squamous intraepithelial lesion (LSIL) cytology, providing useful information for their future clinical management.

## MATERIALS AND METHODS

We consulted international databases such as PubMed, Cochrane Database of Systematic Reviews, EMBASE, Web of Science, with the following terms and their combinations “multiple Papillomavirus infection AND ASCUS”, and “multiple Papillomavirus infection AND LSIL”. We included all available article published until 2020 in English, inclusive retrospective or prospective trials, and reviews. We found 459 records from the preliminary bibliographic search. After the elimination of duplicates and after the excluding the works that were manifestly irrelevant, we carefully examined the 96 most recent or significant papers as it appears below. In particular, we excluded abstracts, letters, and oldest article about multiple HPV infection. For the specific purposes of the present work, we

performed a further selection of the preliminary set of articles, with a more restrictive criterion, *i.e.*, the role of multiple infection in low grade cytology and its clinical application. Finally we consider a total of 15 articles which, in our opinion, represent better the aim of this review (**figure 1**).



**Figure 1.** PRISMA flow chart of research strategy.

## RESULTS

ASCUS and LSIL cytological results are reported in approximately 5% and 2.5% of all cervical Pap smears respectively (3). Recent studies have demonstrated an average HR-HPV positivity rate ranging from 43% to 48.7% in women with ASCUS cytology (4) and from 68% to 82% in women with LSIL cytology (5). The likelihood of CIN2<sup>+</sup> is considered to be 7.4 to 12.6% after ASCUS cytology and 17.4 to 18.6% after LSIL results (6, 7). Nonetheless, previous papers have shown that while CIN2<sup>+</sup> lesions can be identified in 10.7% of women with ASCUS and HR-HPV positivity, they are found in only 1.5% of women with ASCUS and HR-HPV negativity (4). With regards to LSIL cytology, there is evidence that biopsy-diagnosed High-grade Squamous Intraepithelial Lesion (HSIL) is seen in 16% to 26% of HR-HPV positive but in only 4.3% to 13% of HR-HPV negative LSIL pap tests (5). Therefore, these data provide evidence that

HR-HPV positivity is significantly associated with an increase in risk of high-grade cervical lesions in women with ASCUS/LSIL cytology.

Despite the well-known oncogenic risk of HPV16 and HPV18, recent studies provide a new insight in the high prevalence of other HR-HPVs and their significant contribution to a large proportion of high-grade cervical lesions in women with ASCUS/LSIL (8, 9). Wang *et al.*, report that, while based on HPV16/18 model test the rate is only 55.2%, the combination of HPV16/18/31/33/58/52 is able to identify the 93.1% of women with ASCUS cytology but histologically proven CIN2<sup>+</sup> (9). These findings are consistent with Demarco *et al.*, analyses, which confirm that risk of progression differs substantially by HPV type and can be meaningfully categorized into four groups: (1) HPV16, whose cumulative risk of CIN2<sup>+</sup> is 21.5% at 7 years of follow-up; (2) HPV18 and HPV45, whose cumulative risk of CIN2<sup>+</sup> is over 10% at 7 years of follow-up; (3) HPV31;33;52;58;35, whose risk of CIN2<sup>+</sup> is over 5% at 7 years of follow-up; (4) HPV39;51;56;59;68, whose cumulative risk is below 5% at 7 years of follow-up (10).

According to these results, Bonde *et al.*, recommend that ASCUS/LSIL cytology combined with any of HPV16,31,18,33,45 would merit direct colposcopy referral, whereas ASCUS/LSIL cytology combined with any of HPV35,39,51,56,59,66,68 could be at low risk enough to be followed by a 12-month follow-up retesting regimen (11). In accordance with this, some of our previous studies have demonstrated that in women with low-grade cytological abnormalities HPV16,18,31,51,52 genotypes contribute to over 90% of histologically-proven high-grade cervical disease (6, 12). Moreover, we found that in women with ASCUS/LSIL cytology and CIN2<sup>+</sup> histology, the rates of single and multiple infections stratified by each of the most prevalent HPV types were respectively 4.1% and 50.8% for HPV16, 1.6% and 24.9% for HPV18, 4.7% and 29% for HPV31, 2.1% and 16.6% for HPV51, 2.1% and 22.8% for HPV52 (6).

In addition, we observed that HPV infection caused by unknown or untypable genotypes can be detected in 11.7% of cervical cytological samples, and that their prevalence is much significantly higher in women with mild than in those with severe lesions in their Pap smears. Although the risk of high-grade CIN associated with untypable HPV infection is higher than that associated with uninfected patients, it is significantly lower than that associated with known low-risk HPV types. Finally, the low rate of progression to CIN2/3 of subjects with

untypable HPV infection is reassuring and suggests that these women could be followed-up with the same protocol used for HPV negative patients (12). Multiple HPV infections are detected in about 16.7% of women with ASCUS cytology and in 28.7% of LSIL Pap smear results (7). According to literature, the most frequent HR-HPV types found in multiple infections are HPV16/18, HPV18/51, HPV18/52, HPV39/51 and HPV39/52 (13). To date, several studies have reported that 79.5 to 80.8% of CIN2<sup>+</sup> cervical lesions in women with mild cytological abnormalities may harbor multiple HPV infections (6, 14). While only 18.2% of severe cervical lesions are sustained by single HR-HPV infections, 32.7% and 47.2% of them present multiple low/high-risk HPV types and multiple HR-HPV types respectively (6).

Besides, recent studies report that the Odds Ratio (OR) of high-grade CIN among women with multiple compared to single HPV infection is 2.54 (95% CI = 1.70-3.79,  $p < 0.001$ ) (14). These findings confirm the high prevalence of multiple HPV infections in patients undergoing colposcopy because of ASCUS or LSIL, and suggest their potentially significant association with increased risk of CIN2<sup>+</sup> compared to single infections, especially in patients with previous history of SIL/CIN (14). With regards to each cytological outcome, in ASCUS patients the rates of severe biopsy proven lesions are 8.6% and 31.6% in women with single and multiple HPV infections respectively. Similarly, in women with LSIL cytology, the likelihood of CIN2<sup>+</sup> is 12.5% in patients with single and 28.4% in those with multiple HPV infection (6). Hence, these data confirm that compared to subjects with single infection, women with multiple HPV infections have up to four-fold increased risk of CIN2<sup>+</sup>.

Previous research reports that 52% of HPV persistent infections are caused by multiple genotypes. Conversely, 54.8% of samples with multiple HPV infection at baseline show persistent infection during follow-up (13). These results provide further evidence that multiple HPV infections are significantly associated with persistence and, thus, increased oncogenic risk. In addition, our previous studies show that the relative risk of CIN2 and CIN3<sup>+</sup> in women with multiple compared to those with single HPV infection are 2.14 (95% CI = 1.44-3.18) and 2.31 (95% CI = 1.54-3.47) respectively among HPV16-positive patients, and 2.19 (95% CI = 1.3-3.68) and 3.25 (95% CI = 2.29-4.61) in HPV16-negative women (7). It follows that multiple HR-HPV infections are associated

with an increased risk of CIN2 and CIN3+ compared with single HR-HPV infections among women with abnormal cytology, in both HPV16-positive and HPV16-negative subjects, suggesting that multiple genotypes might interact synergistically, increasing the risk of CIN, regardless of HPV16-positivity. According to recent literature, among women with ASCUS/LSIL the rates of histologically-diagnosed CIN3+ are 9% in patients with single HR-HPV infection, 15.9% in subjects with multiple high and low-risk HPV infection, and 20.1% in those with multiple high-risk HPV infection (12). Vice-versa, among women with multiple high and low-risk HPV infection, the prevalence of LSIL and ASCUS cytology is 57% and 16.5% respectively and the rate of histopathological diagnosed CIN2+ is 39% (14). Similarly, among women with multiple high-risk HPV infections, LSIL and ASCUS cytology are reported in 57% and 12% of cases respectively and the rate of CIN2+ at histology is 46% of cases (14). An incremental risk of severe cervical lesions can be observed not only for infections sustained by high-risk strains but also for the association between low and high-risk types. Previous research has shown that in women with abnormal cytological results, the rate of CIN2+ histological lesions is 19.5% in patients with single infection, 31.5% in those coinfecting with 2 genotypes, 57% in those coinfecting with 3 genotypes and 90% in subjects with more than 3 types (14). Hence,

these findings suggest a significant linear trend for increasing severity of cervical lesions and number of co-infecting types.

With respect to clinical practice, several surveys have demonstrated that in severe CIN lesions diagnosed in Loop Electrosurgical Excision Procedure (LEEP) or cold-knife conization, multiple HR-HPV infections are associated with larger cervical lesions as detected by colposcopy (15, 16). Of note, we reported that multiple HPV infections can be detected in 41.2% of subjects with no colposcopic lesions, and 49.8%, 53.4%, 58.1% and 54.7% among women with extension < 25%, 25-50%, 50-75%, > 75% respectively (16). Among women with ASCUS/L-SIL, the Positive Predictive Value (PPV) for CIN2/3 lesions associated with any colposcopic abnormality is higher among patients with multiple than those with single HR-HPV infection, with a linear trend between the extension of colposcopic lesions and the number of high-risk HPVs detected (16). In addition, multiple HPV status and/or HPV16 positivity do not seem to influence the accuracy of colposcopy in the detection of CIN3+ lesions (16). Finally, as we reported in previous papers, among women undergoing conization because of persistent low-grade CIN, multiple HR-HPV infections during follow-up correlate with increased rates of high-grade CIN recurrence (17). **Table I** summarizes the main results.

**Table I.** Main features of frequency and prevalence or relative risk measures of CIN2+ lesions in ASCUS/LSIL patients with different type of multiple HPV infections.

Authors, title, publication year	Design	N° examined patients	Main results
(3) Wright TC <i>et al.</i> Risk detection for high-grade cervical disease using Onclarity HPV extended genotyping in women, ≥ 21 years of age, with ASC-US or LSIL cytology. <i>Gynecol Oncol</i> 2019.	Multicentric clinical trial	33.858 patients	<ol style="list-style-type: none"> <li>1. 5% and 2.5% of PT cytological results are ASCUS and L-SIL respectively.</li> <li>2. In women with ASCUS/L-SIL, the highest risk of CIN2+ is associated with HPV16, followed by HPV31, 18, 33, 58, 52.</li> <li>3. Overall risk for CIN2+ in patients with ASCUS/HR-HPV+ and LSIL/HR-HPV+ is 14,2% and 10,9% respectively.</li> <li>4. If women with ASCUS/LSIL and HPV16, 31, 18, 33/58 are referred to colposcopy, risk for CIN2+ in the remaining HR-HPV positive women drops to only 4,5%.</li> </ol>
(4) Tao X, <i>et al.</i> Atypical squamous cells of undetermined significance cervical cytology in the Chinese population: Age-stratified reporting rates, high-risk HPV testing, and immediate histologic correlation results. <i>Cancer Cytopathol</i> 2020.	Retrospective study	1.597.136 patients	<ol style="list-style-type: none"> <li>1. HR-HPV positivity rate ranges from 43% to 48,7% in women with ASCUS cytology.</li> <li>2. While CIN2+ lesions can be identified in 10.7% of women with ASCUS and HR-HPV positivity, they are found in only 1.5% of women with ASCUS and HR-HPV negativity.</li> </ol>
(5) Rufail M, <i>et al.</i> Low-grade squamous intraepithelial lesion on Papanicolaou test: follow-up rates and stratification of risk for high-grade squamous intraepithelial lesion. <i>J Am Society Cytopathol</i> 2020.	Retrospective review	526 patients	<ol style="list-style-type: none"> <li>1. HR-HPV positivity rate ranges from 68% to 82% in women with L-SIL cytology.</li> <li>2. While CIN2+ lesions can be identified in 16-26% of women with L-SIL and HR-HPV positivity, they are found in only 4.3-13% of women with L-SIL and HR-HPV negativity.</li> <li>3. Higher rates of CIN2+ are found in ASCUS/HR-HPV+ women who have already had previous abnormal PT results (23%).</li> </ol>

Authors, title, publication year	Design	N° examined patients	Main results
(6) Spinillo A, <i>et al.</i> Multiple human papillomavirus infection and high grade cervical intraepithelial neoplasia among women with cytological diagnosis of atypical squamous cells of undetermined significance or low grade squamous intraepithelial lesions. <i>Gynecol Oncol</i> 2009.	Single-centered clinical trial	1.218 patients	<ol style="list-style-type: none"> <li>1. The prevalence of high-grade CIN is 12,6% in patients with ASCUS and HR-HPV positivity and 17,4% in patients with L-SIL and HR-HPV positivity.</li> <li>2. In women with ASCUS/LSIL cytology and CIN2+ histology, the rates of single and multiple infections stratified by each of the most prevalent HPV types are respectively 4.1% and 50.8% for HPV16, 1.6% and 24.9% for HPV18, 4.7% and 29% for HPV31, 2.1% and 16.6% for HPV51, 2.1% and 22.8% for HPV52.</li> <li>3. Overall, the rates of multiple infections are 22.5% among women with negative histology, 63.6% and 79.5% in patients with CIN1 and CIN2+ respectively.</li> <li>4. Among women with ASCUS/L-SIL, the risk of CIN2+ is of 18.7% in patients with single HR-HPV positivity, 32.7% in patients with multiple L/HR-HPV infections, 47.2% in patients with multiple HR-HPV infections.</li> <li>5. After excluding infections by HPV16/18, the risk of CIN2+ in patients with ASCUS and single or multiple HPV infection is 8.6% and 31.6% respectively; the risk of CIN2+ in patients with L-SIL cytology and single or multiple infection is 12.5% and 28.4% respectively.</li> <li>6. Increasing number of HPV types is linearly associated with increasing severity of colposcopic/pathologic outcome.</li> </ol>
(7) Spinillo A, <i>et al.</i> Multiple human papillomavirus infection with or without type 16 and risk of cervical intraepithelial neoplasia among women with cervical cytological abnormalities. <i>Cancer Causes and Control</i> 2014.	Cross-sectional study	3.842 patients	<ol style="list-style-type: none"> <li>1. Multiple HPV infections are detected in 16.7% of women with ASCUS cytology and 28.7% of women with L-SIL cytology.</li> <li>2. Infections by multiple HR-HPVs increase the risk of CIN3+ in both HPV16-positive and HPV16-negative subjects.</li> <li>3. Coinfections with low-risk HPV types does not modify the risk of progression to precancerous cervical lesions associated with high-risk types.</li> </ol>
(8) Song F, <i>et al.</i> Type-specific distribution of cervical hrHPV infection and the association with cytological and histological results in a large population-based cervical cancer screening program: Baseline and 3-year longitudinal data. <i>J Cancer</i> 2020.	Prospective observational study	10.186 patients	<ol style="list-style-type: none"> <li>1. High-risk HPV positivity among women with abnormal cytology is 59.3%, which is 7.4 times greater than that among women with normal cytology.</li> <li>2. The base-line risk for CIN2+ is 25% and 27,3% in women with ASCUS/HPV16 and L-SIL/HPV16 respectively, 7.1% in women with ASCUS/HPV18 (NA in women LSIL/HPV18), 8.4% and 15.5% in women with ASCUS/Other HRHPVs and L-SIL/ Other HRHPVs respectively.</li> </ol>
(9) Wang Y, <i>et al.</i> The efficiency of type-specific high-risk human papillomavirus models in the triage of women with atypical squamous cells of undetermined significance. <i>Cancer Manag Res</i> 2020.	Single-centered clinical trial	34.532 patients	<ol style="list-style-type: none"> <li>1. In women with ASCUS identified as having CIN2+, the HR-HPV infection rate is 97.7%, and the prevalence rates of HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, -66 and -68 are 48.3%, 8.0%, 6.9%, 4.6%, 1.1%, 2.3%, 3.4%, 3.4%, 26.4%, 1.1%, 17.2%, 2.3%, 0.0% and 0.0%, respectively.</li> <li>2. The HPV16/18/31/33/52/58 model shows a higher sensitivity [93.1 (87.8-98.4)], specificity [73.0 (70.7-75.4)], PPV [18.0 (14.5-21.5)], NPV [99.4 (98.9-99.9)], PLR [3.7 (3.1-3.8)] and NLR [0.06 (0.03-0.18)] for the triage of ASCUS patients, as well as colposcopy referral rate (30.9%) is significantly lower than that of the recommended HR-HPV model (44.0%).</li> </ol>
(10) Demarco M, <i>et al.</i> A study of type-specific HPV natural history and implications for contemporary cervical cancer screening programs. <i>EclinicalMedicine</i> 2020.	Longitudinal observational study	11.573 patients	<p>Risk of progression can be stratified into 4 groups basing on HPV type:</p> <ol style="list-style-type: none"> <li>1. HPV16, whose cumulative risk of CIN2+ is 21.5% at 7 years of follow-up;</li> <li>2. HPV18 and HPV45, whose cumulative risk of CIN2+ is over 10% at 7 years of follow-up;</li> <li>3. HPV31;33;52;58;35, whose risk of CIN2+ is over 5% at 7 years of follow-up;</li> <li>4. HPV39;51;56;59;68, whose cumulative risk is below 5% at 7 years of follow-up.</li> </ol>
(11) Bonde JH, <i>et al.</i> Clinical Utility of Human Papillomavirus Genotyping in Cervical Cancer Screening: A Systematic Review. <i>J Lower Gen Tract Dis</i> 2020.	Systematic review	240.674 patients	<p>Applying US threshold for colposcopy of 5.2%:</p> <ol style="list-style-type: none"> <li>1. ASCUS/LSIL cytology combined with HPV16,31,18,33,45 would merit direct colposcopy referral.</li> <li>2. ASCUS/LSIL cytology combined with HPV35,39,51,56,59,66,68 could be followed by a 12-month follow-up retesting regimen.</li> </ol>

Authors, title, publication year	Design	N° examined patients	Main results
(12) Spinillo A, <i>et al.</i> Untypable human papillomavirus infection and risk of cervical intraepithelial neoplasia among women with abnormal cervical cytology. J Med Virol 2014.	Prospective observational study	4.258 patients	<ol style="list-style-type: none"> <li>1. Among women with ASCUS/LSIL cytology and CIN2-3 histology, HPV16,52,32,18,51 are found in the 45.9%, 26.2%, 25%, 14.7%, 14.3% of cases respectively.</li> <li>2. Untypable HPVs can be detected in 11.7% of PT smears, with higher prevalence in women with low-grade cytologic lesions.</li> <li>3. Rates of CIN associated with untypable HPVs are higher than those associated with uninfected samples, but lower than those associated with low-risk HPV types.</li> <li>4. Infection by untypable HPVs shows low rates of progression to high grade CIN.</li> <li>5. Rates of CIN3+ in patients with ASCUS/L-SIL are of 0.4% in HPV negative women and 1.7%, 2.5%, 15.9%, 9%, 20.1% in women infected by untypable HPVs, single/multiple L-R types, multiple L/H-R types, single H-R types and multiple H-R types respectively.</li> </ol>
(13) Oyervides-Muñoz MA, <i>et al.</i> Multiple HPV Infections and Viral Load Association in Persistent Cervical Lesions in Mexican Women. Viruses 2020.	Prospective hospital case study	294 patients	<ol style="list-style-type: none"> <li>1. 52% of HPV persistent infections are caused by multiple genotypes.</li> <li>2. 54.8% of samples with multiple HPV infection at baseline show persistent infection during follow-up.</li> </ol>
(14) Bello BD, <i>et al.</i> Cervical infections by multiple human papillomavirus (HPV) genotypes: Prevalence and impact on the risk of precancerous epithelial lesions. J Med Virol 2009.	Prospective observational study	1.323 patients	<ol style="list-style-type: none"> <li>1. Multiple high-risk HPV infection is associated with the same incremental risk of severe cervical lesions as multiple high/low-risk HPV infection.</li> <li>2. The rate of CIN2+ histological lesions is 19.5% in patients with single infection, 31.5% in those coinfecting with 2 genotypes, 57% in those coinfecting with 3 genotypes and 90% in subjects with more than 3 types.</li> </ol>
(15) Spinillo A, <i>et al.</i> Multiple Papillomavirus Infection and Size of Colposcopic Lesions Among Women With Cervical Intraepithelial Neoplasia. J Low Genit Tract Dis 2016.	Case series	898 patients	In CIN lesions diagnosed by LEEP or cold-knife conization, multiple HR-HPV infections correlate with larger cervical lesions as detected by colposcopy.
(16) Spinillo A, <i>et al.</i> Diagnostic accuracy of colposcopy in relation to human papillomavirus genotypes and multiple infection. Gynecol Oncol 2014.	Cohort study	2526 patients	<ol style="list-style-type: none"> <li>1. In patients with ASCUS/L-SIL, multiple HPV infections are detected in 41.2% of subjects without colposcopic lesions, and 49.8%, 53.4%, 58.1%, 54.7% among subjects with extension &lt; 25%, 25-50%, 51-75%, &gt; 75% respectively.</li> <li>2. Linear trend between the extension of colposcopic lesions and the number of HR-HPVs detected.</li> <li>3. Among women with ASCUS/L-SIL, the PPV for CIN3+ lesions associated with any colposcopic abnormality is 23.5% and 15.5% in subjects with multiple and single infections respectively.</li> <li>4. The accuracy of colposcopy to detect CIN2-3+ is not influenced by neither multiple HPV or HPV16 infections.</li> </ol>
(17) Spinillo A, <i>et al.</i> Outcome of Persistent Low-Grade Cervical Intraepithelial Neoplasia Treated With Loop Electrosurgical Excision Procedure. J Low Genit Tract Dis 2016.	Prospective observational study	252 patients	Among women undergoing conization because of persistent low-grade CIN, multiple HR-HPV infections during follow-up correlate with increased rates of high-grade CIN recurrence.

## DISCUSSION

Co-infection with multiple genotypes of HPV is commonly observed among women with abnormal cervical cytology, as it is reported in 20-50% of cases according to literature (18, 19). Guidelines on the management of low-grade cytological lesions in Western countries are mainly based on the use of HPV DNA test as intermediate triage and direct colposcopy referral for HR-HPV positive ASCUS/LSIL cohorts. A large number of surveys have

claimed that HR-HPV positivity is significantly associated with an increase in risk of high-grade cervical lesions in women with ASCUS/LSIL cytology (4, 5). Recent research shows that, although HPV testing increases the sensitivity and negative predictive value of screening programs, the specificity declines compared with cytology, due to the high prevalence of HPV infection in sexually active women. As a consequence, most women with HR-HPV positive ASCUS/LSIL who undergo colposcopy do not have high-grade cervical lesion. There

is a clinical need to develop risk-stratification approaches that would further reduce the number of unnecessary colposcopy exams for women with ASCUS/LSIL while ensuring that most women with CIN2<sup>+</sup> receive appropriate treatment.

Several studies claim that significant clustering patterns of HPV types and species may occur in multiple infections. Indeed, noteworthy interactions were found both at inter-species level, with higher rates of co-infection by  $\alpha 7$ - $\alpha 9$ - $\alpha 10$ ,  $\alpha 6$ - $\alpha 9$ ,  $\alpha 7$ - $\alpha 10$ , and at inter-genotype level, with higher risk of co-infection by types HPV31-35-56, HPV16-18, HPV51-52. Besides,  $\alpha 9$  species and HPV16 genotype are most frequently represented (1). There is evidence from literature that HPV16 genotype tends to cluster with viral types from different species, as proved by the high rates of co-infection between HPV16 and HPV45 (from  $\alpha 9$  and  $\alpha 7$  species respectively), while co-infection with other  $\alpha 9$ - genotypes is quite uncommon, suggesting possible competitive interactions among genotypes from the same species at a cellular level (20). Nonetheless, other studies on multiple HPV infection have reported that individual types and species associate at random based on their relative frequency (21, 22).

The above-mentioned heterogeneity of results also depends on socio-demographic and behavioural reasons, such as the origin and the characteristics of the studied population, their socio-economic status (20), young age, HIV seropositivity and recent sexual intercourse (6, 23). As far as racial influences on HPV genotype distributions are concerned, recent literature (24) reports that, despite the higher proportion of HPV<sup>+</sup> ASCUS cytology in black than in white women, the prevalence of HPV16 type is higher in white (12.7%) than in black (7.8%) patients. Conversely, black women show a higher proportion of other high-risk HPVs (HPV 31,33,35,39,45,51,52,56,58,59,66,68) than white ones (47% vs 38.1%).

Several cross-sectional studies suggest that multiple HR-HPV infections are associated with an increased risk of severe CIN (14). Namely, it has been observed a linear trend between the number of viral types involved and the severity of lesion (1, 20, 14). Multiple HR-HPVs increases the risk of CIN2<sup>+</sup> in both HPV16-positive and HPV16-negative subjects, suggesting a potential synergistic interaction between HR-HPVs, favoring the progression of CIN lesions (25). Co-infection with low-risk HPV types, although quite common, does not seem to modify the risk of precancerous cervical lesions associated with the high-risk viral strains involved (25). None-

theless, other reports disprove multiple HPV infection as a significant risk factor for severe cervical lesions, claiming that any single area of CIN is due to the action of a single carcinogenic type, while other high-risk HPVs detected in cervical smears are related to independent transient infections (26, 27).

Van der Marel J. *et al.*, claim that women with High-Grade CIN and concomitant multiple HPV infection, often present with various heterogeneous cervical lesions, each one caused by a single carcinogenic type though, as proved by Laser Capture Microdissection – Polymerase Chain Reaction (LCM-PCR) genotyping system (26).

*In vitro* studies have recently proved that coinfection of a single cell with more than one HPV type is possible, and that this could result in significant inter-genotype molecular interactions, affecting the life cycle of the single viral types involved, as well as their own ability to persist and to drive cell transformation (28, 29). In this particular case, Biryukov *et al.*, have observed that coinfection with HPV16 and HPV18 decreases HPV18 E1 and E4 transcription, that is a proxy for a significant decrease in HPV18 infectivity compared to a single infection (28). Indeed, these results indicate that there is some degree of inter-genotypic competition or superinfection exclusion, for which HPV16 is able to partially block or interfere with HPV18 life cycle. Besides, it is important to consider that HPV16 and HPV18 show different binding localization patterns and internalization times. In fact, while HPV16 is able to bind directly to the cell surface and be internalized, HPV18 needs to bind to the Extracellular Matrix (ECM) prior to conformational changes and transfer to the cell surface, where L2 is then cleaved by furin in order to enter the cell. Because of these different attachment requirements, HPV16 shows higher cell entry rates than HPV18. Hence, they hypothesized that superinfection exclusion might occur early during the attachment/entry phase of the viral life cycle, suggesting that HPV16 minor capsid L2 protein may play a crucial role in blocking furin-dependent HPV18 cleavage and, thus, decreasing HPV18 infectivity (28). On the other hand, Mori S. *et al.* suggest that the inter-genotypic competition observed in the coinfection of a single cell with HPV16 and HPV18 may be due to a mechanism of genome replication interference as a result of the transcription of heterooligomers composed of HPV16/18 helicase E1 Oligomerization-Domain (OD), which are responsible for a decrease in viral replication rates. This could possibly be explained

by the lower efficiency of chimeric proteins in binding to the DNA replication origin, recruiting cellular factors required for HPV replication and transcription and unwinding double-stranded DNA (29).

Previous analyses have shown that multiple HR-HPV infection correlates significantly with greater period of infection, which in turn stands for enhanced risk of progression (13).

Hence, it is important to note that the reported molecular mechanisms of inter-genotypic competition mostly occur in the early phases of acute viral infection. On the contrary, it has been observed that in cells that harbour persistent HR-HPV infections, there is no block in the ability of a second viral type to superinfect cells (28).

These findings in biology are consistent with the clinical counterpart for which it has been observed that the detection of a new additional viral type in the setting of a previously diagnosed HR-HPV persistent infection increases the risk of CIN2<sup>+</sup> if compared to simultaneous coinfection (14). Therefore, it is conceivable that significant genotype-specific clustering patterns might mostly be represented in CIN lesions of women infected with multiple HR-HPV types, based on their own ability to give rise to persistent infections.

Since persistent high-risk HPV infection is a prerequisite of cervical dysplasia and cancer, recent primary prevention programs, based on type-specific high-risk HPV vaccination, have led to a significant decrease in CIN1-CIN3 cervical lesions in the vaccinated cohorts (30). In fact, while Gardasil (4vHPV, Gardasil, Merck, 2006) and Cervarix (2vHPV, Cervarix, GlaxoSmithKline, 2009), raising immunity against HPV6-11-16-18 and HPV16-18 respectively, are protective against high-risk HPV type 16 and 18 which are responsible for 70% of all cervical dysplasia, Gardasil 9 (9vHPV, Gardasil 9, Merck, 2015) extends protection also from HPV 31-33-45-52-58, which are responsible for another 18.3-20% of cervical neoplasia (30). Nonetheless, the above mentioned preventive vaccines, which trigger the production of L1-specific neutralizing antibodies, are strictly type-specific. Moreover, it has been shown that the preventive efficacy of HPV vaccination programs seems to decrease in women over 25 who have been previously infected by targeted high-risk HPVs (31), although they would still benefit to a certain extent (32). Interestingly enough, the recent prospective case-control study Speranza (32) has reported a decrease of 81.2% in the risk of H-SIL recurrence during four-year follow up time after LEEP procedure in patients undergoing tetra-

lent HPV vaccination 30 days after the intervention. These results are in accordance with previous studies, such as Future I and II and Patricia, which claim the clinical efficacy of HPV vaccination after LEEP in decreasing the risk of disease recurrence (33). As well as protecting patients from *de novo* infections by targeted HR-HPVs, HPV vaccination after LEEP is supposed to prevent the reduction of immune response against Human Papillomavirus that seems to follow escissional procedures (32). Indeed, recent research (34, 35) suggests that patients with persistent HR-HPV infection show higher levels of TNF $\alpha$  in their cervical fluids. However, it has been observed a decline in cervical TNF $\alpha$  levels after escissional procedures, leading to a cervical microenvironment similar to that of HPV-naïve patients (32). Hence, it has been hypothesized that HR-HPV vaccination boosts neutralizing antibody production by memory B-cells from the basement membrane, enhancing their titres up to 100-1000 times and, therefore, preventing any virus in the surgical site from entering regenerating epithelial cells (32).

Hence, the introduction of type-specific HR-HPV vaccination brings along with it significant clinical implications in primary and secondary prevention programs, as well as redrawing prevalence and distribution of the main oncogenic viral types, based on which new screening risk-stratification tools should be considered.

## CONCLUSIONS

In conclusion, this review provides further evidence that multiple HR-HPV infection is a significant risk factor for severe cervical lesions in women with ASCUS and LSIL cytology, and highlights that increased oncogenic risk might be strictly associated with peculiar type-specific profiles, independent of HPV-16. Besides, it offers interesting insights in the epidemiology of HPV genotype distribution, especially in the post-vaccination era, suggesting new risk-stratification models to be taken into account. These observations could offer useful guidance on proper clinical management of women with mild cytological abnormalities.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## New needs for menopausal women during COVID-19 pandemic. A physician-based investigation

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### ABSTRACT

It was evaluated whether the COVID-19 pandemic may have influenced the manifestation of menopausal symptoms. In a web interview, 143 physicians, experts on the menopause, were asked to rate their perceived prevalence of hot flush, sleep disorder, sexual disturbance, anxiety and depression as 'rare', 'seldom', 'frequent' and 'very frequent' in patients they managed prior to and during the COVID-19 pandemic. According to physicians, there was no major change in the prevalence of hot flush, sleep disorder and sexual disturbance but prevalence of anxiety (39.8% vs 75.5%;  $p < 0.0001$ ) and depressive (35.6% vs 72.0%;  $p < 0.0001$ ) symptoms increased during COVID-19 pandemic. COVID-19 pandemic has changed the composite picture of menopausal symptoms, challenging physicians to adjust their therapeutic approach to the new need. Approaches capable to treat vasomotor symptoms but also highly effective on mood disturbance should be preferred.

### SOMMARIO

Lo studio ha voluto valutare se le manifestazioni della sintomatologia climaterica fossero cambiate durante la pandemia da COVID-19. Lo studio è stato effettuato durante un webinar su 143 specialisti esperti nel trattamento dei disturbi menopausali. È stato chiesto di definire, in relazione al periodo pre-COVID e al periodo pandemico, la prevalenza nelle loro pazienti di vampate di calore, disturbi del sonno, disturbi della sessualità, ansietà e sintomi depressivi, secondo le categorie "rara", "talvolta", "frequente" e "molto frequente". Dai dati emerge che mentre la prevalenza delle vampate di calore, disturbi del sonno e della sessualità non si è modificata, la prevalenza dei disturbi dell'ansia (39.8% vs 75.5%;  $p < 0.0001$ ) e della depressione (35.6% vs 72.0%;  $p < 0.0001$ ) è aumentata durante la pandemia. Il cambiamento nella prevalenza dei sintomi menopausali richiede un adattamento terapeutico alle nuove necessità e la scelta di presidi che siano in grado non solo di curare i sintomi vasomotori ma anche di avere un effetto positivo sul tono dell'umore.

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### Key words

*Menopause; COVID-19; anxiety; depression; psychological symptoms; vasomotor symptoms; hot flush; climacteric.*

## INTRODUCTION

Hot flushes are the most evident and frequent symptom of menopause (1, 2) but menopausal symptoms are not limited to vasomotor instability and are related to many functions of the brain, and peripheral organs and apparatus (3-11). Taken together, the disturbances making up menopausal symptoms, as evaluated by climacteric scales, are mainly based on menopause-related mood changes (12, 13) that impact a woman's daily relationships with others, her perceptions and activities. Everyday life has changed remarkably during the COVID-19 pandemic. Social and family contacts have been reduced (14), there are fewer work opportunities and salaries have dropped (15), access to healthcare facilities has been hampered (16, 17), and our future financial well-being and health along with that of our families is uncertain (18). All these factors may be having a negative impact on our psychological equilibrium (19, 24), and this could be particularly evident in menopausal women, who are already living a critical period of their lives (5, 17, 22, 23). In these women, the COVID-19 pandemic may change the prevalence of symptoms and the composite picture of menopausal complaints. New needs and requests may emerge, challenging physicians to find novel therapeutic approaches specifically tuned to meet them. In this investigation, we evaluated whether physicians have experienced change in the prevalence of symptoms and request of treatment among their menopausal patients during the COVID-19 pandemic.

## MATERIALS AND METHODS

An investigation was performed during a webinar offering an update on menopause management during the COVID-19 pandemic. The webinar was supported by the Italian Society of Menopause and was delivered to 143 participants, all members of the Italian Society of Menopause with specific experience on menopause. Prior to the start of the session, physicians were asked to answer 5 questions investigating their perception of the frequency of menopausal symptoms that women usually complained about prior to the COVID-19 pandemic. Questions were specific for hot flushes, sleep disorders, anxiety, depression and sexual disturbances. For each of the 5 symptoms, physicians were given 10 seconds to categorize their perceived prevalence of these

symptoms in their patients as 'rare', 'seldom', 'frequent', or 'very frequent'. They were then asked to concentrate on patients they were managing during the COVID-19 pandemic. The same 5 questions were resubmitted with the same procedure.

The results obtained were elaborated almost immediately and made available graphically for discussion. Data were then tabulated. The number of physicians rating the perceived prevalence of each single symptom in the pre- and in the COVID-19 era as 'rare', 'seldom', 'frequent' and 'very frequent' was calculated. Physicians rating a symptom prevalence as 'rare' and 'seldom' were also considered together in a single group, and those who defined a symptom prevalence as 'frequent' and 'very frequent' in another single group. Data referring to the pre-COVID-19 era and to during the pandemic were compared by contingency tables and the chi squared test.  $P < 0.05$  was considered statistically significant.

## RESULTS

According to the physicians, the prevalence of hot flushes in their patients remained largely unchanged during the COVID-19 pandemic even though more physicians rated prevalence of hot flushes as 'frequent' ( $p < 0.027$ ) and fewer physicians rated this symptom as 'very frequent' ( $p < 0.009$ ) during the pandemic (**table I**). Physicians observed no major change in the prevalence of sleep problems, although a small increase was observed in the number of those rating sleep problems in their patients as 'very frequent' during the COVID-19 pandemic ( $p < 0.035$ ) (**table I**). There was no change in physicians' perception of the prevalence of sexual disturbances in patients before and during the COVID-19 pandemic (**table I**). A higher number of physicians rated prevalence of symptoms of depression in their patients as 'frequent' ( $p < 0.0002$ ) and 'very frequent' ( $p < 0.0001$ ) during the COVID-19 pandemic (**table I**). Similarly, a higher number of physicians rated the prevalence of symptoms of anxiety in their patients as 'frequent' ( $p < 0.008$ ) and 'very frequent' ( $p < 0.0001$ ) during the COVID-19 pandemic (**table I**). When categories 'rare' and 'seldom' were combined in a single group, and 'frequent' and 'very frequent' in another group, physicians rated a similar prevalence of 'frequent/very frequent' for the symptoms hot flushes (93.2% vs 96.0%), sleep

problems (93.6% vs 86.4%), and sexual problems (79.0% vs 79.7%) in patients they managed during than prior to COVID-19 pandemic, respectively (figure 1). More physicians (75.8% vs 39.8%, respectively;  $p < 0.0001$ ) observed their patients suffered from 'frequent/very frequent' anxiety symptoms during the COVID-19 pandemic than before it (figure 1). Similarly, more physician (72.0% vs 35.6%, respectively;  $p < 0.0001$ ) observed their patients suffered from 'frequent/very frequent' symptoms of depression during the COVID-19 pandemic than before it (figure 1).

## DISCUSSION

The COVID-19 pandemic has induced a new way of life around the world, characterized by social restraints, loneliness, reduced support, occupational uncertainties, fear of the disease, fear for the health of relatives, and continuous negative input from the media (14-18). Several reports indicated that all these factors have an impact on psychological equilibrium, increasing disturbances, particularly anxiety and symptoms of depression (19-24). An increase in anxiety and symptoms of depression has also been reported as a manifestation of menopausal symptoms (1, 5). Prevalence is usually lower and less defined than that of classic menopausal

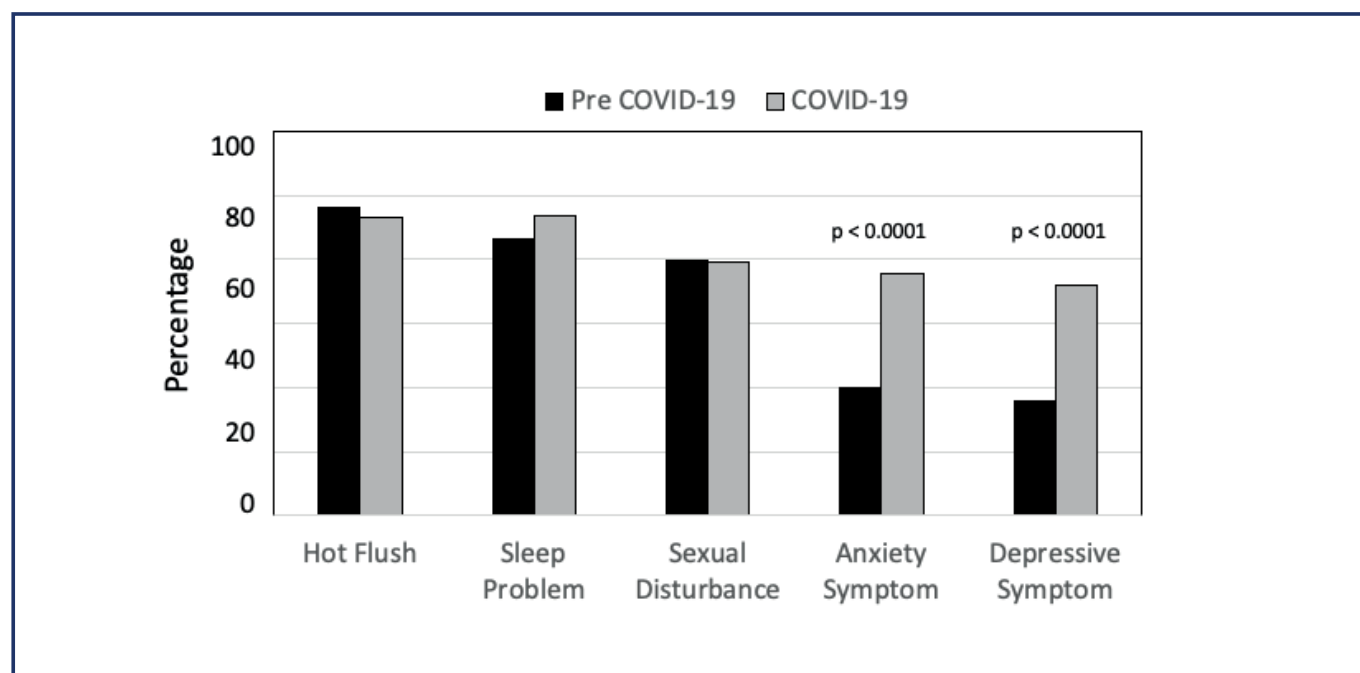
symptoms such as hot flushes (1). Thus, most of the therapies and remedies for women with menopausal symptoms are directed to improve hot flushes (11, 25-29).

Current investigation indicates that according to physicians, who are used to managing menopausal symptoms, the prevalence of anxiety and symptoms of depression has increased significantly during the COVID-19 pandemic, to reach the prevalence of hot flushes. This modification in the composite picture of menopausal symptoms has posed greater therapeutical challenges. Position statements and guidelines (11, 25-29) have given suggestions on hormonal and non-hormonal treatments for menopausal symptoms but have failed to indicate how to manage mood changes, even if these represent a major component of climacteric scales evaluating menopausal symptoms and quality of life (12, 13). Mood disturbances increase in the menopausal transition (1, 5) and hormone therapy was reported to improve anxiety and symptoms of depression (5). Yet its effect may not be sufficiently powerful in conditions of elevated psychological disturbance.

The same applies for non-hormonal remedies that have been studied mainly to improve vasomotor symptoms (29). Physicians are now requested to give greater consideration to mood alterations and to become more familiar with the manage-

**Table I.** Percentage of physicians (n = 143) who rated as 'rare', 'seldom', 'frequent' or 'very frequent' the prevalence of menopausal symptoms in women evaluated prior to or during the COVID-19 pandemic.

	Rare (%)	Seldom (%)	Frequent (%)	Very frequent (%)
<b>Hot flushes</b>				
Pre-COVID-19	0.7	6.3	28.7	67.3
During COVID-19	0	7.7	41.2	52.0
p	0.317	0.643	0.027	0.009
<b>Sleep problems</b>				
Pre-COVID-19	2.1	10.4	64.7	21.7
During COVID-19	0.7	6.3	60.8	32.8
p	0.314	0.210	0.495	0.035
<b>Sexual problems</b>				
Pre-COVID-19	1.4	18.9	48.2	31.5
During COVID-19	2.8	19.6	40.5	38.5
p	0.409	0.881	0.190	0.215
<b>Anxiety symptoms</b>				
Pre-COVID-19	4.9	55.2	36.3	3.5
During COVID-19	0.7	17.5	52.0	23.8
p	0.031	0.0001	0.008	0.0001
<b>Depressive symptoms</b>				
Pre-COVID-19	12.6	51.7	32.8	2.8
During COVID-19	4.2	23.8	54.5	17.5
p	0.010	0.0001	0.0002	0.0001



**Figure 1.** Percentage of physicians ( $n = 143$ ) who rated as 'frequent' or 'very frequent' the prevalence of different menopausal symptoms in women evaluated prior to and during the COVID-19 pandemic.

ment of these increasingly frequent yet often neglected disturbances; here, remedies also capable of re-establishing psychological equilibrium could be more useful. Therapeutical choices should now be directed mainly to pharmacological (30) or non-pharmacological (31-33) remedies capable not only to treat vasomotor symptoms but also to concomitantly improve mood.

A major limitation of this investigation is that it was not performed with the use of psychological scales. However, it involved physicians used to treating symptoms of the menopause, who perceived new demands and were requested to give new answers to women's needs. They observed that COVID-19 has altered the composite picture of menopausal symptoms.

## CONCLUSIONS

The change in the manifestation of menopausal symptoms, during COVID-19 pandemic, has posed a renewed challenge and the need to adapt therapeutic approaches to the new emerging requests.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## Healthy Foetuses – Misoprostol Insert Induction (HF-MIND): a multicentre Italian study on the misoprostol vaginal insert for induction of labour

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### ABSTRACT

**Objective.** To evaluate the efficacy and safety of the misoprostol vaginal insert in a selected cohort of healthy mothers and foetuses at late-term gestation or with premature rupture of membranes.

**Methods.** Retrospective data were collected by 12 centres that previously developed and shared the same protocol for the induction of labour with the misoprostol vaginal insert. Pregnant women at late-term gestation or with premature rupture of membranes with an unfavourable cervix were recruited. To be eligible, maternal and foetal well-being was assessed: absence of medical and/or obstetrical maternal complications, normal foetal growth (abdominal circumference > 10 percentile, AFI > 5 cm, absence of notch in uterine arteries Doppler velocimetry), category I foetal heart rate and, in case of PROM, normal white blood cell count and PCR. Subgroup analyses were performed for parity, status of membranes and BMI < 30 / > 30.

### SOMMARIO

**Obiettivo.** Valutare l'efficacia e la sicurezza dell'inserto vaginale di misoprostolo in una coorte selezionata di madri e feti sani oltre termine o con rottura prematura delle membrane.

**Metodi.** I dati retrospettivi sono stati raccolti da 12 centri che in precedenza avevano sviluppato e condiviso lo stesso protocollo per l'induzione del travaglio con l'inserto vaginale di misoprostolo. Sono state reclutate donne in gravidanza oltre termine o con rottura prematura delle membrane con cervice sfavorevole. Per l'idoneità è stato valutato il benessere materno e fetale: assenza di complicanze mediche e/o ostetriche materne, normale crescita fetale (circonferenza addominale > 10 percentile, AFI > 5 cm, assenza di notch nelle arterie uterine velocimetria Doppler), tracciato cardiocografico categoria I e, in caso di PROM, normale conta leucocitaria e PCR. Le analisi dei sottogruppi sono state eseguite per parità, stato delle membrane e BMI < 30 / > 30.

**Results.** The median time-to-delivery was 14:07 hours:minutes (hh:mm; range: 10:37-21:10). A total of 256 (62.6%) women delivered within 24 hours from the insertion of the device. Uterine tachysystole occurred in 59 women (14.4%), and tocolysis was used in only 14 patients (3.4%). High maternal BMI > 30 (OR = 0.92; 95% CI 0.86-0.99;  $p < 0.03$ ), multiparity (OR = 0.48; 95% CI 0.22-0.96;  $p < 0.05$ ) and PROM (OR = 0.49; 95% CI 0.26-0.86;  $p < 0.02$ ) were significantly associated with a lower risk of tachysystole. The Caesarean section rate was 22%, and an emergency Caesarean section was performed in 12 patients (2.9%). Neonatal pH was < 7.00 in 4 newborns (1%), and 8 newborns (2%) were admitted to the NICU. No uterine rupture, maternal or neonatal deaths occurred.

**Conclusions.** In a selected cohort of healthy mothers and foetuses at late-term or with PROM with unfavourable cervix, the misoprostol vaginal insert proved its efficacy for the induction of labour that achieved an effective reduction in time-to-delivery, a reduction in other augmentation interventions, and a low rate of Caesarean section without increasing obstetrical and foetal complications.

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## INTRODUCTION

Induction of labour (IOL) is an increasingly common procedure in clinical obstetrics that can sometimes put strain on women, gynaecologists, and obstetric units. On one hand, IOL can cause greater pain and women can experience additional anxiety (1). On the other hand, gynaecologists may find themselves dealing with complications such as uterine hyperstimulation and precipitous delivery or vice versa a failure of the procedure itself.

A recent systematic review showed that a low dose (< 50 µg) of titrated oral misoprostol solution had the lowest probability of Caesarean section and was associated with a good efficacy profile, whereas vaginal misoprostol (≥ 50 µg) had the highest probability of achieving a vaginal delivery within 24 hours (2). Subsequently, the same author produced a ranking of both pharmacological and mechanical methods for the IOL, finding that misoprostol and oxytocin with amniotomy were more successful than other agents in achieving vaginal delivery within 24 hours (3). In 2014, Mysodelle, a new vaginal insert

**Risultati.** Il tempo medio al parto è stato di 14:07 ore:minuti (hh:mm; intervallo: 10:37-21:10). Un totale di 256 (62,6%) donne ha partorito entro 24 ore dall'inserimento del dispositivo. La tachisistolia uterina si è verificata in 59 donne (14,4%) e la tocolisi è stata utilizzata solo in 14 pazienti (3,4%).

BMI materno elevato > 30 (OR = 0,92; 95% CI 0,86-0,99;  $p < 0,03$ ), multiparità (OR = 0,48; 95% CI 0,22-0,96;  $p < 0,05$ ) e PROM (OR = 0,49; 95% CI 0,26 -0,86;  $p < 0,02$ ) erano significativamente associati a un minor rischio di tachisistole. Il tasso di taglio cesareo è stato del 22% e in 12 pazienti (2,9%) è stato eseguito un taglio cesareo d'urgenza. Il pH neonatale era < 7,00 in 4 neonati (1%) e 8 neonati (2%) sono stati ricoverati in terapia intensiva neonatale. Non si sono verificate rotture uterine, decessi materni o neonatali.

**Conclusioni.** In una coorte selezionata di madri e feti sani oltre termine o con PROM con cervice sfavorevole, l'inserto vaginale di misoprostolo ha dimostrato la sua efficacia per l'induzione del travaglio che ha ottenuto un'efficace riduzione del tempo al parto, una riduzione di altri interventi di potenziamento del travaglio e un basso tasso di taglio cesareo senza aumentare le complicanze ostetriche e fetali.

## Key words

*Misoprostol vaginal insert; induction of labour; tachysystole; unfavourable cervix; PROM.*

containing 200 mcg of misoprostol (MVI), was licensed and approved for IOL. In a pivotal study, in comparison with the dinoprostone vaginal insert (DVI), MVI showed a faster action, shortening the time-to-delivery with a comparable safety profile in terms of the Caesarean section rate and neonatal outcomes (4). However, an increase in uterine tachysystole and subsequent tocolysis was observed. The implications of these adverse effects have been investigated in a post hoc study that revealed that managing cases of uterine tachysystole with foetal heart rate (FHR) abnormalities was more challenging for MVI than for DVI due to the longer half-life of misoprostol (5). However, MVI retrieval due to adverse effects did not increase neonatal intensive care unit (NICU) admissions compared with those for women whose inserts were removed for other reasons.

Recently, the European Medicines Agency advised that MVI can cause uterine tachysystole, which might not respond to tocolytic treatment (6). This fact has placed more emphasis on the use of MVI, leading to the publication of several observational

studies that compared MVI with misoprostol by vaginal or oral routes or with dinoprostone (7-12). All of these studies, however, had the limitation of considering rather small samples of patients (fewer than 200 subjects for a single treatment arm to small cohorts of 50 subjects (13)) with different indications for IOL, including intrauterine growth restriction (IUGR) and preeclampsia.

Regarding efficacy, all but one reported studies showed a reduction in the time-to-delivery and a higher rate of vaginal birth within 24 hours for MVI (11). However, concerns and disagreements on the safety profile of MVI were reported, especially with regard to higher tachysystole rates, Caesarean section rates and neonatal outcomes. These safety concerns were greater in cohorts with a large number of pathological pregnancies (9). We hypothesized that a multicentre study could help to overcome the need for a large cohort: this study is the result of a retrospective analysis of clinical data observed in our 12 centres that developed and shared a single protocol focused on the IOL of healthy mothers and healthy foetuses in order to better understand the safety issues posed by this new device.

## MATERIALS AND METHODS

### *Design of the study*

Starting in February 2016, 13 Italian obstetric units discussed, developed and then shared a common clinical management procedure for IOL with MVI for pregnant women who were late-term or had premature rupture of membranes (PROM), with no foetal or maternal risk factors according to the following protocol. On September 2018, these centers agreed to conduct a retrospective analysis starting with data from October 2016 to August 2018. The participant centers are listed in **table I**. The beginning date was chosen to let each center test the clinical feasibility of this protocol. Eight months were considered long enough to incorporate this protocol as one of the clinical skills for each staff member in all centers. This study was reported to the ethical committees as per local regulations.

### *Patients*

Inclusion criteria were pregnant women admitted for IOL at late-term pregnancy (41 + 0 to 41 + 6 (weeks + days) of gestation) or at term with PROM

**Table I.** List of participant centers.

Participant centers
1. San Pietro Hospital FBF, Rome, Italy
2. Buzzi Children's Hospital, ASST FBF Sacco, University of Milan, Milan, Italy
3. ASST Valle Olona, Gallarate and Busto Hospital, Gallarate and Busto Arsizio, Varese, Italy
4. ASST Spedali Civili, University of Brescia, Brescia, Italy
5. San Raffaele Hospital, Milan, Italy
6. Poliambulanza Foundation, Brescia, Italy
7. San Gerardo Hospital - FMBBM, Monza, Italy
8. ASST- Garda, Desenzano del Garda, Italy
9. Policlinico San Pietro- I.O.B. San Donato Group, Ponte San Pietro, Bergamo, Italy
10. Carlo Poma Hospital, Mantova, Italy
11. ASST Rhodense, Rho Hospital, Rho, Milan, Italy
12. Careggi Hospital, University of Florence, Florence, Italy
13. IRRCS Ca' Granda Foundation, Mangiagalli Hospital, University of Milan, Milan, Italy

(> 37 + 0 (weeks + days) of gestation), with an unfavourable cervix (assessed by a simplified Bishop score < 4), and the following well-being characteristics: foetal abdominal circumference > 10<sup>th</sup> percentile, amniotic fluid index > 5 cm, absence of notch in uterine arteries Doppler velocimetry, and category I FHR tracing (14). Exclusion criteria were multiple pregnancies, chromosomal or structural foetal abnormalities, previous caesarean section and any positive medical history or complications of pregnancy as hypertensive disorders and insulin dependent diabetes. Women with PROM with PCR > 1.5 mg/dl or white blood cell count > 15.000/μl were excluded from this study.

### *Methods*

Data were collected in real time for each patient from all centers on the same standard case report form. Written consent for the induction of labour was obtained from patients before the insertion of MVI. FHR monitoring was performed every 4-6 hours depending on the presence of uterine activity. Indications for MVI removal were as follows: more than 24 hours interval from the insertion, onset of labour or presence of regular symptomatic contractions for > 60 min without cervical dilatation > 3 cm, tachysystole or hypertonus, and changes in FHR tracing (ACOG category II or III). Tachysystole was defined as more than five contractions per 10 min for at least 20 min, and uterine hypertonus was defined as a single contraction lasting at least 2 min

(15). Management of these complications involved, first, conservative manoeuvres and, second, tocolysis with ritodrine or atosiban. To reduce overstimulation, MVI was removed in presence of regular symptomatic contractions for > 60 min. If spontaneous expulsion occurred, the device was not replaced with a new MVI. Oxytocin administration, if required, was permitted 30 min after the removal of the vaginal insert. When the Bishop score was persistently low (< 5) after the use of MVI, an attempt to continue with the induction process was made with prostaglandin E2 or Cook double balloon<sup>®</sup>, amniotomy and/or oxytocin per local protocol. Induction failure was defined as the absence of regular uterine activity after any attempt with induction methods accepted by the patient.

Data recorded included baseline demographic and obstetric characteristics, labour and delivery data and neonatal outcomes.

The primary efficacy outcomes included the median time-to-delivery from the beginning of induction and intrapartum oxytocin use. The primary safety outcomes included the emergency Caesarean section rate, the incidence of tachysystole and hypertonus, arterial pH < 7 and foetal admission to a NICU.

### Statistical analysis

For maternal characteristics and study outcomes, descriptive statistics were used. Time-to-delivery was presented graphically using Kaplan-Meier curves. Using Student's t-test for continuous variables and Fisher's exact test for categorical variables, subgroup analyses were performed for nulliparous *vs* pluriparous women, PROM *vs* intact membranes and BMI < 30 *vs* ≥ 30 at term. Main demographic and obstetric characteristics were evaluated as predictors for tachysystole with a univariate linear regression analysis. Parameters with a

significance level of up to  $p = 0.05$  were included in a multivariate regression model. Data analysis was performed with Excel and R 3.5.1 software.

## RESULTS

A total of 409 women were included in the analysis. Demographic characteristics and indications for the induction of labour are presented in **table II**, according to parity. The main study outcomes are summarized in **table III**.

### Main efficacy outcomes

The median time at insert removal from the beginning of induction was 8:40 hours:minutes (hh:mm; IR 5:55-13:00). The most frequent indication for removal was onset of labour (67.5%), followed by tachysystole or hypertonus (14.4%), exceeding 24 hours (7.3%), FHR monitoring category II or III (6.3%) and spontaneous expulsion (4.4%). Additional induction methods were used in only 8 women (1.9%). Induction failure definitively occurred in 9 women (2.2%).

Vaginal delivery within 24 hours occurred in significantly more multiparous than nulliparous women (74 (74.7%) *versus* 182 (56.9%)  $p = 0.004$ ). Similarly, time-to-delivery from the beginning of induction was significantly shorter in multiparous women (**figure 1**).

No differences were found in the median time-to-delivery between women with PROM and those with intact membranes or between women with BMI >30 *vs* those with BMI < 30 at term (**figure 2**).

IOL in women with PROM was associated with a higher rate of epidural analgesia (66.0% *vs* 53.8%, respectively,  $p = 0.02$ ) and oxytocin administration (47.5% *vs* 27.5%, respectively,  $p = 0.0001$ ) and a lower incidence of tachysystole (9.9% *vs* 17.4%,

**Table II.** Demographic characteristics and indication for induction of labour.

	Total (409)		Nulliparous (310)		Multiparous (99)		p value
Maternal Age (years) <sup>*</sup>	32.7	± 5.4	32.5	± 5.5	33.4	± 5.3	0.16
BMI <sup>*</sup>	28.2	± 4.1	28.0	± 3.9	28.7	± 4.5	0.12
Gestational Age (weeks) <sup>*</sup>	40.5	± 1.2	40.4	± 1.3	40.8	± 0.9	0.008
Late-term gestation <sup>†</sup>	247	60.4%	182	58.7%	65	65.7%	0.16
PROM <sup>‡</sup>	162	39.6%	128	41.3%	34	34.3%	0.23
Bishop score <sup>§</sup>	2	(1-3)	2	(0-3)	2	(1-3)	< 0.001

<sup>\*</sup>Data presented as mean ± standard deviation; <sup>†</sup>data presented as numbers and percentage; <sup>‡</sup>data presented as median and interquartile range; <sup>§</sup>P value obtained for comparison between nulliparous and multiparous by Fisher exact test for categorical variables and Student t tests for continuous variables.

**Table III.** Main study outcomes in the total population and according to parity.

Labour and delivery	Total (409)		Nulliparous (310)		Multiparous (99)		p value
Oxytocin for labour acceleration*	145	35.5%	119	38.4%	26	26.3%	0.03
Median time to vaginal delivery hh:mm (range)#	14:07 (10:37-21:10)		14:41 (11:01-22:10)		13:12 (9:54-17:48)		0.04
Operative vaginal delivery (vacuum)*	61	14.9%	50	16.1%	11	11.1%	0.26
Caesarean Section*	90	22.0%	77	24.8%	13	13.1%	0.017
Median time from MVI removal to CS#	4:51 (1:49-9:04)		5:02 (1:57-8:36)		4:40 (0:50-9:54)		0.85
Any Tachysystole*	59	14.4%	49	15.8%	10	10.1%	0.19
Tachysystole with FHR involvement*	14	3.4%	13	4.2%	1	1.0%	0.20
Hypertonus*	9	2.2%	9	2.9%	0		0.12
Tocolysis*	14	3.4%	12	3.9%	2	2.0%	0.53
Meconium-stained fluid (3 <sup>rd</sup> degree)*	33	8.1%	24	7.7%	9	9.1%	0.67
<b>Maternal outcomes</b>							
PPH >1000 CC*	17	4.2%	11	3.5%	6	6.1%	0.26
3 <sup>rd</sup> degree perineal tear*	6	1.9%	5	2.1%	1	1.2%	1
<b>Neonatal outcomes</b>							
Neonatal birth weight (kg) <sup>o</sup>	3.5	± 0.39	3.4	± 0.39	3.6	± 0.37	0.0009
APGAR < 7 at 5 min*	4	1.0%	3	1.0%	1	1.0%	1
pH < 7.0*	4	1.0%	3	1.0%	1	1.0%	1
pH < 7.10*	36	8.8%	24	7.7%	12	12.1%	0.22
ABE < - 12*	16	3.9%	11	3.5%	5	5.1%	0.55
Admission to NICU*	8	2.0%	6	1.9%	2	2.5%	1

\*Data presented as numbers and percentage; #data presented as median and interquartile range; <sup>o</sup>data presented as mean ± standard deviation; p value obtained for comparison between nulliparous and multiparous by Fisher exact test for categorical variables and Student t tests for continuous variables.

respectively,  $p = 0.04$ ) vs IOL in women with intact membranes.

Women with PROM showed a non-significant reduction in the Caesarean section rate in comparison with women with intact membranes (18.5% vs 24.3%, respectively,  $p = 0.18$ ). A non-significant reduction was also observed in women with BMI < 30 regardless of the state of the membranes (20.9% vs 24.8%, respectively,  $p = 0.28$ ).

### Main safety outcomes

Uterine tachysystole occurred in 59 women, but tocolysis was performed in only 14 of these patients. The main indication for Caesarean section was an abnormal FHR pattern (58.9%), followed by dystocia-arrest of cervical dilatation (22.2%). An emergency Caesarean section was performed in 12 patients (2.9%), and five of these cases were suspected placental abruptions. All women in which placental abruption occurred belonged to the first recruited half of our sample and most of them (4) experienced episodes of tachysystole or hypertonus. Maternal characteristics and obstetrical factors were investigated by univariate and multivariate analyses as predictors of tachysystole. Only high maternal BMI > 30 (OR = 0.92; 95% CI 0.86-0.99;  $p$

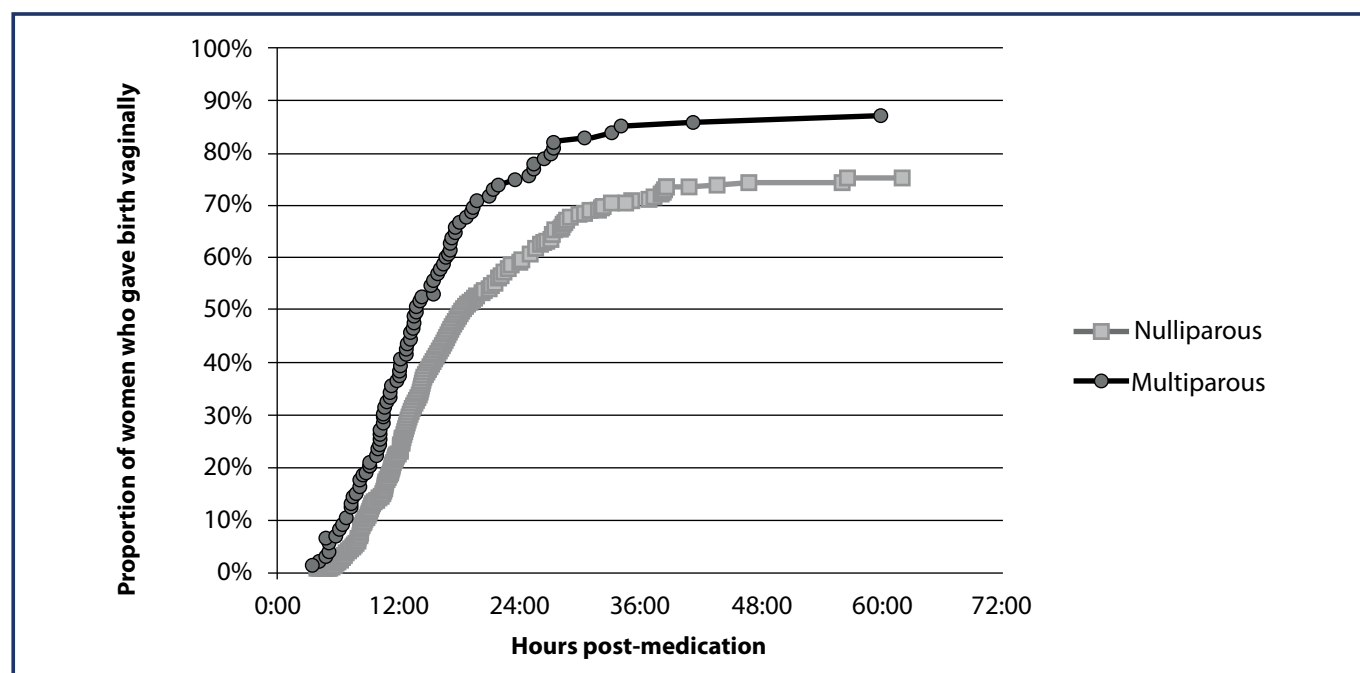
< 0.03), multiparity (OR = 0.48; 95% CI 0.22-0.96;  $p < 0.05$ ) and PROM (OR = 0.49; 95% CI 0.26-0.86;  $p < 0.02$ ) were significantly and independently associated with a lower risk of tachysystole.

Maternal and neonatal outcomes are shown in **table III**. No significant differences in these outcomes were detected between PROM and intact membranes or between BMI > 30 and BMI < 30. No uterine rupture, maternal or neonatal deaths occurred in this study.

### DISCUSSION

The results of this present study confirm the efficacy of MVI for inducing labour in pregnant women who are late term or who have PROM with an unfavourable cervix without additional maternal or foetal risks.

The median time-to-vaginal delivery in this cohort, even with lower oxytocin use, was better than that in the Expedite trial (21 hours and 40%, respectively) (4). In our study, mean BMI was lower and the prevalence of IOL in PROM was higher than those reported by the Expedite trial; both of these maternal characteristics were associated with favourable induction outcomes and could have contributed to



**Figure 1.** The Kaplan-Meier plot shows the complete data of the time-to-delivery for nulliparous and multiparous women.

this outcome (16, 17). Kaplan-Meier plots in **figure 2 a, b** confirmed this trend in our cohort. Recent studies by Marsdal and Mayer, which included women with a mean BMI < 30 and a percentage of PROM > 20%, in agreement with our results, reported a median time to vaginal delivery between 12 and 15 hours (7, 11).

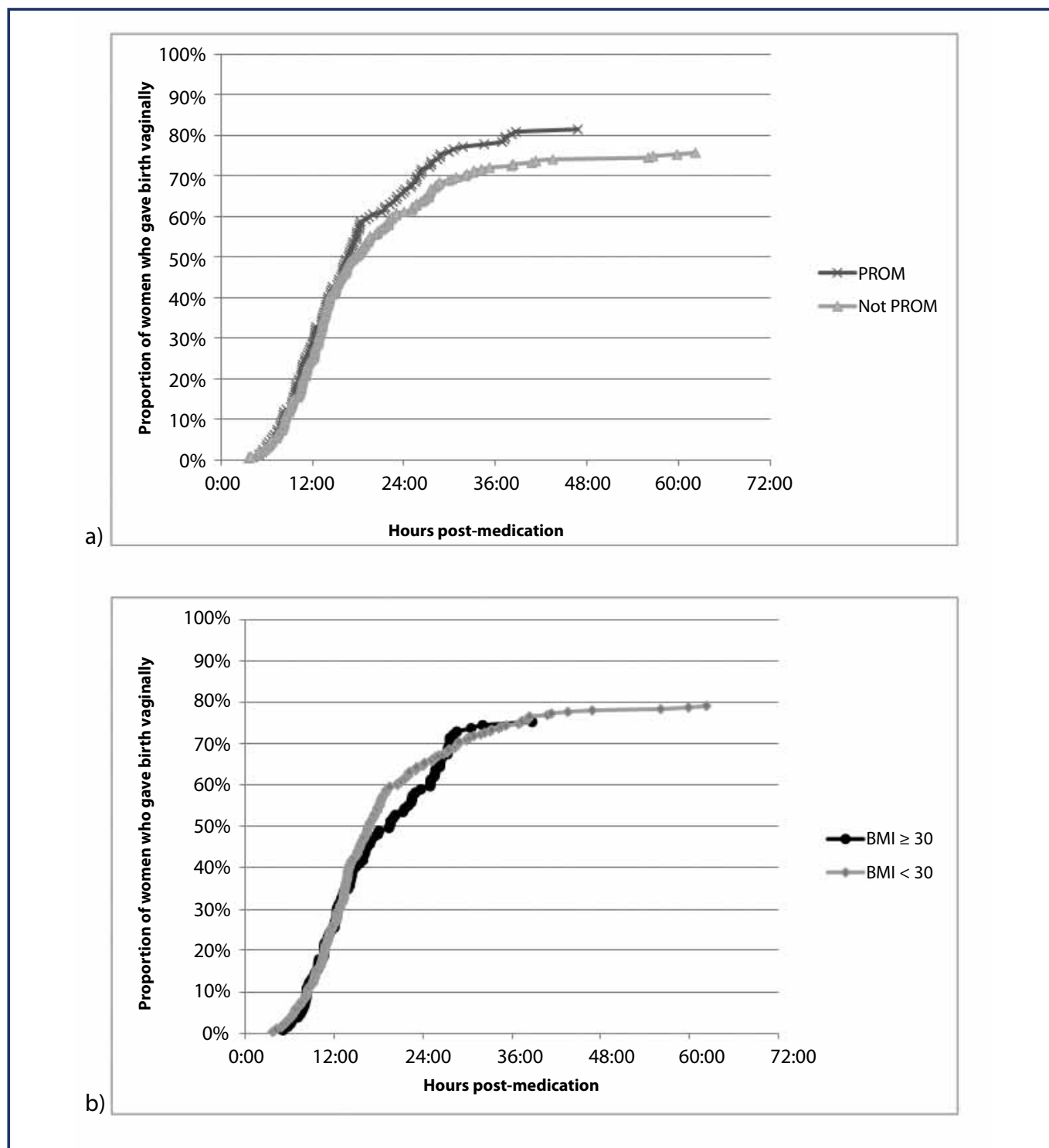
The safety profile observed in our study should be analysed in light of the design of our shared multi-centre protocol. We chose to select among patients undergoing IOL those with maternal and foetal well-being, both at late-term gestation or after PROM. These criteria resulted from the analysis of reported cohorts that confirmed a relatively high prevalence of tachysystole (7-13). Healthy foetuses are more likely to overcome these transient episodes of poor oxygenation, thereby reducing the risk of emergency Caesarean section.

The rate of Caesarean sections was in fact lower than that reported in the Expedite trial (22% vs 26%, respectively) and in other studies with a smaller number of cases, such as the study by Dobert (4, 9). This latter study included also IUGR and pre-eclampsia as indications for IOL and reported 47% tachysystole and 39% Caesarean section rates. The Caesarean section rate observed in our cohort was also lower than that reported by Redling (29.7%) in a smaller cohort in which IUGR and gestational diabetes had been excluded (10). An even lower rate of Caesarean sections (11.8%) was recently reported

by Marsdal in a sample with 86 pregnant women who underwent IOL, half of whom had a pre-induction with cervical balloon (7). These contradictory results should be interpreted with caution given the small number of patients and different recruitment criteria.

In our cohort, half of Caesarean sections were performed for an abnormal FHR pattern, but only 2.9% occurred as emergency Caesarean sections. However, among the first half of the cases recruited, we observed five Caesarean sections for placental abruption. These data did not emerge in other previous studies and should be emphasized with caution since the association with uterine hyperstimulation. In our cohort, the rate of tachysystole was three times lower than that reported by Wing *et al.* and was still high compared to those obtained with other pharmacological and mechanical induction methods (3, 4). Tocolysis was performed in only 3.4% of patients, while other studies reported higher rates between 16.8% and 38.5% (9, 11).

Excessive uterine activity has been associated with foetal acidosis at birth because of inadequate uterine relaxation time, reduced perfusion pressure and impaired oxygen delivery to the placenta (18). Stewart *et al.* found no differences in infant outcomes when tachysystole occurred during early labour, despite FHR decelerations being associated with an increasing number of contractions (19). However, considering over 50,000 deliveries, Heuser *et al.* found that



**Figure 2.** The Kaplan-Meier plot shows the complete data of the time-to-delivery for two subgroups: women with PROM vs intact membranes (a) and women with BMI > 30 vs BMI < 30 at term (b).

tachysystole increased the chance of composite neonatal morbidity (20). In that multivariable analysis, significant risk factors associated with tachysystole were the use of oxytocin and misoprostol, epidural analgesia, and hypertension, while multiparity was associated with a decreased risk of tachysystole. No differences in the incidence of placenta abrupt-

tion were found between women who experienced tachysystole and those who did not. Bolla *et al.* failed to identify any predictors of tachysystole after MVI administration, while we found a significant risk reduction for high maternal BMI, multiparity and PROM (8). A reason could be that the mean baseline BMI and percentage of PROM

were lower in that study than in our study (mean BMI 22.8 vs 27.7, PROM 9.5% vs 39.6%, respectively). To understand the direct effect of tachysystole on safety outcomes, we decided both to exclude conditions such as preeclampsia and IUGR and to include only foetuses that met composite criteria for well-being.

This allowed us to observe better foetal outcomes than those of the Expedite study while maintaining a non-negligible percentage of NICU admission of 2% and 4 cases of neonatal pH < 7.0 (1%). However, the percentages of admission to NICU as well as the percentages of Caesarean sections were not significantly different from those observed in a large cohort of IOL with 25 mg of sublingual misoprostol (21).

The validity of the present study is limited to healthy mothers and foetuses. In addition, there is no internal comparison with other induction methods. Evaluating data progressively over time, we noted that most adverse events, including placental abruption, occurred in the first half of recruited patients, which addresses the need for adequate clinical experience.

## CONCLUSIONS

In a selected cohort of healthy mothers and foetuses at late-term gestation or with PROM, the MVI could represent an efficient method for the IOL. When this method is applied to patients with these characteristics, the efficacy profiling of MVI regarding the time-to-delivery, the reduction of augmentation interventions, and the low rate of Caesarean section can be exploited without additional risks of obstetrical emergencies or foetal compromise.

## CONTRIBUTIONS

All authors contributed to the design of the study and to data collection and discussion of results and commented and improved the manuscript.

Ferrazzi, Brembilla, Urban contributed to data analysis.

Ferrazzi and Brembilla contributed in preparing the draft manuscript.

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## CONFLICT OF INTERESTS

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## NR2F6, LOXL2 and DMBT1 expression in cervical cancer tissues, prognostic and clinicopathological implications

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### ABSTRACT

**Background.** Detection of novel predictive markers for early detection of lymph nodes metastases in cervical cancer is very important. Disturbed NR2F6 expression was found in many cancers playing different roles according to cancer type. LOXL2 was incriminated in cancer progression and unfavorable survival in many cancer types. Decreased DMBT1 expression was found in many cancers during their progression.

Aim of the present work was to assess expression of NR2F6, LOXL2 and DMBT1 in cervical cancer tissues using immunohistochemistry then correlating their expression with grade and stage of the tumor, occurrence of lymph nodes and distant metastases in addition to evaluating their prognostic roles.

**Materials and methods.** We assessed the expression of NR2F6, LOXL2 and DMBT1 in samples retrieved from sixty two cervical cancer patients in cancer tissues and corresponding adjacent normal tissues and we correlated markers expression with clinical data, pathological findings and patients' prognosis.

**Results.** High expression of NR2F6, LOXL2 and low expression of DMBT1 was up-regulated in cervical cancer tissues more than in adjacent non-neoplastic cervical tissues and was associated with high grade ( $p = 0.005$ ), lymphovascular space involvement, advanced FIGO stage, resistance to chemotherapy, tumor recurrence, and shorter survival rates ( $p < 0.001$ ).

**Conclusions.** Up regulation of NR2F6, LOXL2 and down regulation of DMBT1 were associated with unfavorable pathological parameters, bad clinical findings and dismal outcome of cervical cancer patients.

### SOMMARIO

**Contesto.** La rilevazione di nuovi marcatori predittivi per la diagnosi precoce delle metastasi linfonodali nel cancro cervicale è molto importante. L'espressione distorta di NR2F6 è stata trovata in molti tumori che svolgono ruoli diversi a seconda del tipo di cancro. LOXL2 è stato incriminato nella progressione del cancro e nella sopravvivenza sfavorevole in molti tipi di cancro. La diminuzione dell'espressione di DMBT1 è stata trovata in molti tumori durante la loro progressione. Lo scopo del presente lavoro era valutare l'espressione di NR2F6, LOXL2 e DMBT1 nei tessuti del cancro cervicale mediante immunocistochemica, quindi correlando la loro espressione con il grado e lo stadio del tumore, la presenza di linfonodi e metastasi a distanza, oltre a valutare i loro ruoli prognostici.

**Materiali e metodi.** Abbiamo valutato l'espressione di NR2F6, LOXL2 e DMBT1 in campioni prelevati da sessantadue pazienti con cancro cervicale nei tessuti tumorali e nei corrispondenti tessuti normali adiacenti e abbiamo correlato l'espressione dei marcatori con i dati clinici, i risultati patologici e la prognosi dei pazienti.

**Risultati.** L'alta espressione di NR2F6, LOXL2 e la bassa espressione di DMBT1 erano sovraregolate nei tessuti del cancro cervicale più che nei tessuti cervicali adiacenti non neoplastici ed erano associate ad alto grado ( $p = 0,005$ ), coinvolgimento dello spazio linfovaskolare, stadio FIGO avanzato, resistenza a chemioterapia, recidiva del tumore e tassi di sopravvivenza più brevi ( $p < 0,001$ ).

**Conclusioni.** La sovraregolazione di NR2F6, LOXL2 e la sottoregolazione di DMBT1 sono state associate a parametri patologici sfavorevoli, risultati clinici negativi e risultati negativi dei pazienti con cancro cervicale.

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### Key words

*Cancer cervix; NR2F6; LOXL2; DMBT1; prognosis, immunohistochemistry.*

## INTRODUCTION

Cancer cervix is the 4<sup>th</sup> commonest cancer and 4<sup>th</sup> cause of malignancy associated fatalities in women worldwide (1). Squamous cell carcinoma of the cervix is the commonest histopathological subtype of cancer cervix forming about 75-80% of all diagnosed cervical carcinoma cases (2).

Staging of cervical cancer is performed preoperatively (3). Presence of lymph node metastasis is the most important predictive factor of cancer recurrence and dismal outcome in cervical cancer (4). Therefore, detection of novel predictive markers for early detection of lymph nodes metastases in cervical cancer is very important.

Nuclear receptor subfamily 2 group F member 6 (NR2F6) is mapped on chromosome 19p13.1.1; and it encodes a conserved 43-kDa protein (5). NR2F6 was found to be responsible for regulating many biological processes as organogenesis and cellular differentiation during embryogenesis (6). Disturbed NR2F6 expression was found in many cancers playing different roles according to cancer type (7, 8). Lysyl oxidase-like 2 (LOXL2) is a lysyl oxidase (LOX) family member that plays an essential role in making cross-links of collagen and elastin in the extracellular matrix (9). LOXL2 was incriminated in cancer progression and unfavorable survival in many cancer types (10). Additionally, LOXL2 was found to be one of the recent therapeutic targets for cancer treatment (11).

Deleted in malignant brain tumor 1 (DMBT1), which is mapped on chromosome 10q25.3-26.1, was primarily discovered in glioblastoma and medulloblastoma as deleted, hence responsible for their progression and dismal outcome (12).

Recently, decreased DMBT1 expression was found in many cancers during their progression (13, 14) NR2F6, LOXL2 and DMBT1 expression in cervical cancer was not sufficiently clarified.

Aim of the present work was to assess expression of NR2F6, LOXL2 and DMBT1 in cervical cancer tis-

sues using immunohistochemistry then correlating their expression with grade and stage of the tumor, occurrence of lymph nodes and distant metastases in addition to evaluating their prognostic roles.

## MATERIALS AND METHODS

The present prospective study was performed on 62 cervical cancer patients where we acquired 124 specimens from both malignant cervical cancer tissues and corresponding adjacent normal tissues. Patients who were admitted and operated in Gynecology and Obstetrics Department and General Surgery Department, Faculty of Medicine, Zagazig University. Samples were processed and evaluated in Pathology Department, Faculty of Medicine, Zagazig University and patients were followed up for 5 years in Clinical Oncology and Nuclear Medicine Department and in Medical Oncology Department in the period from January 2013 to May 2018.

All included samples were stained with NR2F6, LOXL2 and DMBT1 using immunohistochemistry. We assessed the relationship between NR2F6, LOXL2 and DMBT1 tissue protein expression with disease progression and survival outcome of included cervical cancer patients.

The inclusion criteria were:

1. patients with a sure diagnosis of cervical squamous cell carcinoma and adenocarcinoma with accurate staging,
2. patients with complete clinical and pathological data and
3. patients with complete records of about 30 months follow-up.

Exclusion criteria were:

1. patients with other histopathological subtypes of cervical cancer,
2. patients with preoperative administration of radiotherapy, chemotherapy or hormonal therapy and
3. inoperable patients.

We acquired consents from included patients and an ethical approval for performing the study from the local ethical committee of Faculty of Medicine, Zagazig University.

### Immunohistochemical (IHC) analysis

Primary antibodies used were: primary rabbit polyclonal anti- NR2F6 antibody (ab137496), anti-LOXL2 antibody (ab96233) and DMBT1 (MyBioSource, MBS9416387). Human gastric carcinoma tissue, human esophageal cancer tissue and non-neoplastic mucosa of the colon were used as positive controls for NR2F6, LOXL2 and DMBT1 respectively. IHC analysis was done as previously described (15).

### Evaluation of IHC of NR2F6, LOXL2 and DMBT1

We assessed nuclear NR2F6 expression, cytoplasmic LOXL2 expression and cytoplasmic and membranous DMBT1 expression in cervical cancer tissues and adjacent non-neoplastic cervical mucosa. Evaluation of the stain was done according to staining extent and intensity.

The extent was scored as follows: 0 (negative tumor cells); 1 (< 10% positive tumor cells); 2 (10%–50% positive tumor cells); 3 (> 50% positive tumor cells). The intensity was scored as follows: 0, negative; 1, weak stain (light yellow); 2, moderate stain (yellow brown); 3, strong stain (brown). The final stain index was calculated by multiplying scores of extent and intensity, yielding results from 0–9. The suitable cut-off value was 4. Values above that value are considered high expression and below this value were considered low expression.

### Statistical analysis

All statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL, USA).

The relationship between NR2F6, LOXL2 and DMBT1 expression and the clinicopathologic features of cervical cancer was analyzed using the chi-square test and Fisher's exact test. We used Spearman's rank correlation coefficients to calculate the bivariate correlations between the studied variables. We plotted survival curves using the Kaplan–Meier method and compared them using log-rank testing. Relative risk ratios were calculated using the Cox proportional hazard model. Univariate and multivariate survival distributions were

compared using log-rank testing. We considered  $p < 0.05$  statistically significant.

### NR2F6 expression in cervical cancer and adjacent non-neoplastic tissues

Tissue protein expression of NR2F6 was up-regulated in cervical cancer tissues more than in adjacent non-neoplastic cervical tissues and was negative in all samples of non-neoplastic cervical mucosa ( $p < 0.001$ ) (**table I**).

High expression of NR2F6 was present in 34 (54.8%) of cervical cancer tissues and was associated with

**Table I.** Association between expression of NR2F6, LOXL2 and DMBT1 in cervical carcinoma and adjacent normal mucosa tissues.

	Cervical cancer (N = 62)		Adjacent normal (N = 62)		p-value <sup>†</sup>
	No. (%)	No. (%)	No. (%)	No. (%)	
<b>NR2F6</b>					
Low	28 (45.2%)	62 (100%)	62 (100%)	0	< 0.001
High	34 (54.8%)	0 (0%)	0 (0%)	0	
<b>LOXL2</b>					
Low	28 (45.2%)	50 (80.6%)	50 (80.6%)	12 (19.3%)	0.001
High	34 (54.8%)	12 (19.3%)	12 (19.3%)	50 (80.6%)	
<b>DMBT1</b>					
Low	40 (61.3%)	0 (0%)	0 (0%)	62 (100%)	< 0.001
High	22 (38.7%)	62 (100%)	62 (100%)	0 (0%)	

<sup>†</sup> Chi-square test;  $p < 0.05$  is significant.

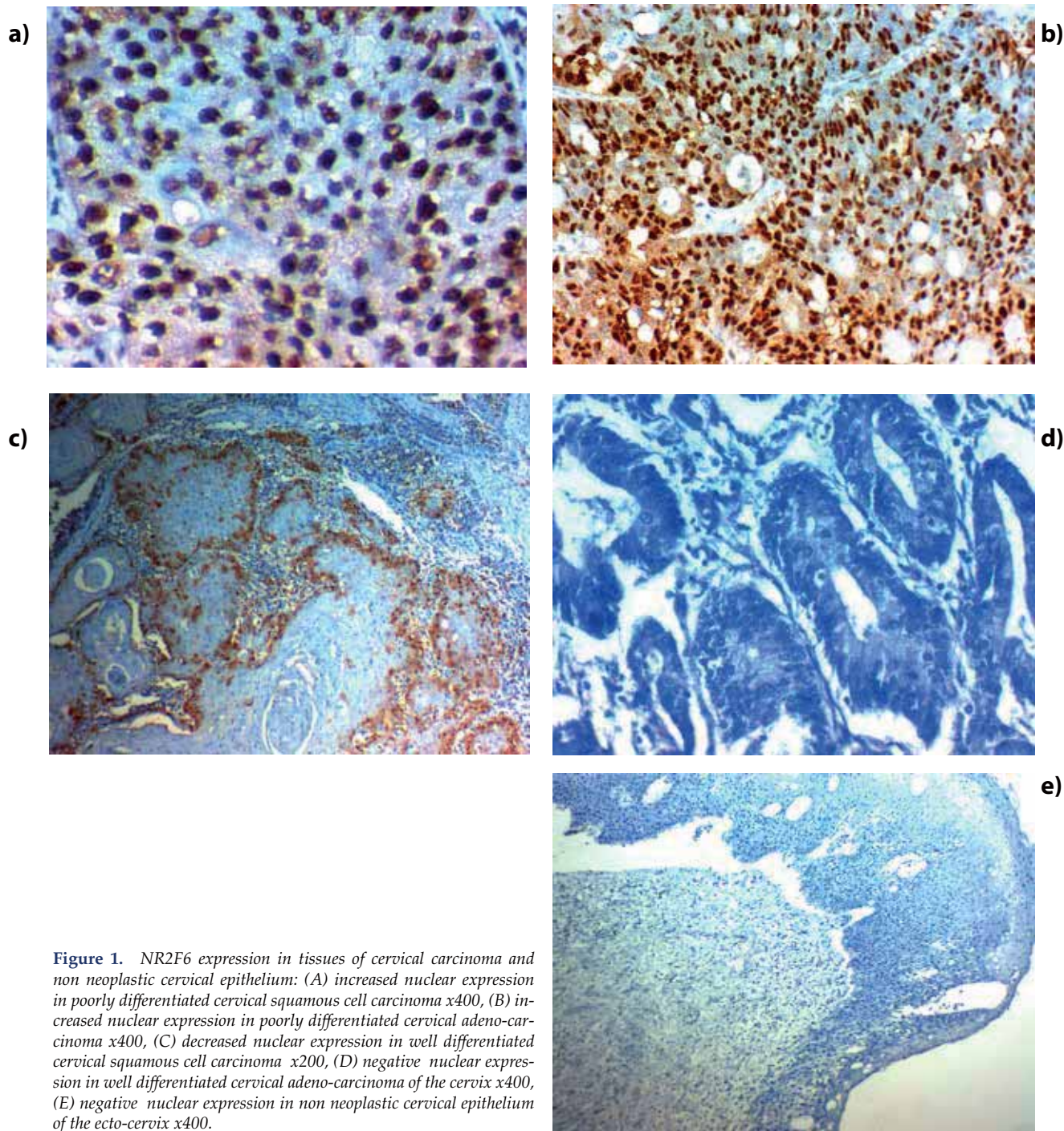
old age of the patient ( $p = 0.002$ ), large tumor size ( $p = 0.004$ ), high grade ( $p = 0.005$ ), lymphovascular space involvement, para-uterine organ infiltration, advanced FIGO stage, resistance to chemotherapy, tumor recurrence ( $p < 0.001$ ).

Patients with high NR2F6 expression had shorter recurrence free survival (RFS) and overall survival (OS) rates ( $p < 0.001$ ) (**figures 1, 4, tables II-V**).

### LOXL2 expression in cervical cancer and adjacent non-neoplastic tissues

Tissue protein expression of LOXL2 was up-regulated in cervical cancer tissues more than in adjacent non-neoplastic cervical tissues ( $p = 0.002$ ) (**table I**).

High expression of LOXL2 was present in 34 (54.8%) of cervical cancer tissues and was associated with old age of the patient ( $p = 0.002$ ), large tumor size ( $p = 0.004$ ), high grade ( $p = 0.005$ ), lymphovascular space involvement, para-uterine organ infiltration, advanced FIGO stage, resistance to chemotherapy, tumor recurrence ( $p < 0.001$ ).



**Figure 1.** NR2F6 expression in tissues of cervical carcinoma and non neoplastic cervical epithelium: (A) increased nuclear expression in poorly differentiated cervical squamous cell carcinoma x400, (B) increased nuclear expression in poorly differentiated cervical adenocarcinoma x400, (C) decreased nuclear expression in well differentiated cervical squamous cell carcinoma x200, (D) negative nuclear expression in well differentiated cervical adenocarcinoma of the cervix x400, (E) negative nuclear expression in non neoplastic cervical epithelium of the ecto-cervix x400.

Patients with high LOXL2 expression had shorter recurrence free survival (RFS) and overall survival (OS) rates ( $p < 0.001$ ) (figures 2, 4, tables II-V).

#### ***DMBT1 expression in cervical cancer and adjacent non-neoplastic tissues***

Tissue protein expression of DMBT1 was markedly down regulated in cervical cancer tissues than in

adjacent non-neoplastic cervical tissues and high expression was found in all samples of non-neoplastic cervical mucosa ( $p < 0.001$ ) (table I).

High expression of DMBT1 was associated with low grade ( $p = 0.049$ ), absence of myometrium invasion ( $p = 0.039$ ), absence of lymphovascular space involvement, absence of para-uterine infiltration, early FIGO stage ( $p = 0.022$ ), response to chemotherapy ( $p = 0.33$ ) and lower incidence of tumor recurrence ( $p = 0.028$ ).

**Table II.** Association between patients demographics, prognostic parameters and expression of NR2F6, LOXL2 and DMBT1 in the cervical cancer patients.

	Total Patients		NR2F6		P*	LOXL2		P*	DMBT1		P*
	N =	%	Low	High		Low	High		Low	High	
			N = 28 (45.2%)	N=34 (54.8%)		N = 28 (45.2%)	N = 34 (54.8%)		N = 40 (61.3%)	N = 22(38.7%)	
<b>Age group:</b>											
≤ 55 years old	25	40.3	26 (92.7)	11 (32.4)	0.002	16 (57.1)	9 (26.5)	0.002	9 (22.5)	16 (72.7)	< 0.001*
> 55 years old	37	59.7	2 (7.1)	23 (67.6)		12 (42.9)	25 (73.5)		31 (77.5)	6 (27.3)	
<b>Histopathology:</b>											
Squamous cell carcinoma	44	71	19 (67.9)	25 (73.5)	0.78	19 (67.9)	25 (73.5)	0.78	28 (70)	16 (72.7)	0.821
Adenocarcinoma	18	29	9 (32.1)	9 (26.5)		9 (32.1)	9 (26.5)		12 (30)	6 (27.3)	
<b>Size:</b>											
< 4 cm	6	9.7	6 (21.4)	0 (0)	0.004	6 (21.4)	0 (0)	0.004	3 (7.5)	3 (13.6)	0.657
≥ 4 cm	56	90.3	22(78.6)	34 (100)		22 (78.6)	34 (100)		37 (92.5)	24 (86.4)	
<b>Grade:</b>											
I	10	16.1	10 (35.7)	0 (0)	0.005	10 (35.7)	0 (0)	0.005	4 (10)	6 (27.3)	0.049
II	38	61.3	17 (60.7)	21 (61.8)		16 (57.1)	22 (64.7)		27 (67.5)	11 (50)	
III	14	22.6	1 (3.6)	13 (38.2)		2 (7.1)	12 (35.3)		9 (22.5)	5 (22.7)	
<b>LVSI:</b>											
Absent	44	71	27 (96.4)	17 (50)	< 0.001*	26 (92.9)	18 (52.9)	0.001*	28 (70)	16 (72.7)	0.022
Present	18	29	1 (3.6)	17 (50)		2 (7.1)	16 (47.1)		12 (30)	6 (27.3)	
<b>Lymph node:</b>											
Absent	30	48.4	22 (78.6)	8 (23.5)	< 0.001*	22 (78.6)	8 (23.5)	0.002*	17 (42.5)	13 (59.1)	0.211
Present	32	51.6	6 (21.4)	26 (76.5)		6 (21.4)	26 (76.5)		23 (57.5)	9 (40.9)	
<b>Distant metastasis:</b>											
Absent	46	74.2	27 (96.4)	19 (55.9)	< 0.001*	20 (71.4)	10 (29.4)	0.003*	29 (72.5)	17 (77.3)	0.681
Present	16	25.8	1 (3.6)	15 (44.1)		8 (28.6)	24 (70.6)		11 (27.5)	5 (27.7)	
<b>Stage:</b>											
I	6	9.7	6 (21.4)	0 (0)	< 0.001*	6 (21.4)	0 (0)	0.001*	3 (7.5)	3 (13.6)	0.286
II	24	38.7	16 (57.1)	8 (23.5)		14 (50)	10 (29.4)		14 (35)	10 (45.5)	
III	16	25.8	5 (17.9)	11 (32.4)		6 (21.4)	10 (29.4)		12 (30)	4 (18.2)	
IV	16	25.8	1 (3.6)	15 (44.1)		2 (7.1)	14 (41.2)		11 (27.5)	5 (22.7)	
<b>NR2F6:</b>											
Low	28	45.2				26 (92.9)	2 (5.9)	<	14 (35)	14 (63.6)	0.03*
High	34	54.8				2 (7.1)	32 (94.1)	0.001*	26 (65)	8 (36.4)	
<b>LOXL2:</b>											
Low	28	45.2	26 (92.9)	2 (5.9)	< 0.001*				13 (32.5)	15 (68.2)	0.007*
High	34	54.8	2 (7.1)	32 (94.1)					27 (67.5)	7 (31.8)	
<b>DMBT1:</b>											
Low	40	61.3	28 (100)	10 (29.4)	< 0.001*	28 (100)	10 (29.4)	<			
High	22	38.7	0 (0)	24 (70.6)		0 (0)	24 (70.6)	0.001*			

\*p < 0.05 is statistically significant; \*Chi square test.

Patients with high DMBT1 expression had longer recurrence free survival (RFS) and overall survival (OS) rates (p < 0.001) (figures 3, 4, tables II-V).

## DISCUSSION

We showed that high NR2F6 expression was found in cancer tissues more than in adjacent non-neoplastic tissues and its high expression was associated with higher grade and advanced stage of cervical cancer. Similarly Niu *et al.*, (1) reported for the first time that high NR2F6 expression is correlated with poor prognosis, unfavorable clinical characteristics and dismal outcome.

NR2F6 might be considered a novel prognostic biomarker and therapeutic target for cervical cancer. NR2F6 was found to be overexpressed and associated with poor prognosis in patients with many cancers (7). These results denoted that it promoted tumor development and progression.

Niu *et al.*, (1) showed that there was upregulation of NR2F6 mRNA and its tissue protein expression in cervical cancer and they have found a significant correlation between its tissue expression and advanced FIGO stage, tumor recurrence, resistance to chemotherapy, and presence of lymph nodes metastases.

Lymph nodes metastases is the most important predictive prognostic parameter and determining

**Table III.** Association between treatment-related results and cervical cancer patients outcome.

	N = 62	%
<b>Treatment:</b>		
Surgery	13	21
Surgery and radiotherapy	10	16.1
Surgery and chemotherapy	17	27.4
Surgery, radiotherapy and chemotherapy	14	22.6
Radiotherapy	4	6.5
Chemotherapy	4	6.5
<b>Response:</b>		
PD	37	59.7
SD	4	6.5
PR	7	11.3
CR	14	22.6
<b>Response:</b>		
OAR	41	66.1
NR	21	33.9
<b>Outcome:</b>		
Alive	37	59.7
Dead	25	40.3
<b>Disease free survival (months) (N = 38):</b>		
Mean ± SD	29.03 ± 6.49	
Range	16 - 36	
<b>Overall survival (months):</b>		
Mean ± SD	27.68 ± 9.18	
Range	10 - 36	

factor for treatment of early-stage cervical cancer (16).

Predicting occurrence of lymph node metastases before treatment of cervical cancer allows selecting the best management options (1). In the current study we found that NR2F6 was highly expressed in cervical cancer with positive lymph nodes metastases so it could be considered a predictive biomarker for lymph nodes metastases in early stage cervical cancer.

We also found that increased tissue protein expression was related to poor survival of patient hence considering it as a novel prognostic biomarker.

Despite discovering the values of using cancer cervix screening programs using Pap smear in early diagnosis of cancer cervix which in turn led to improvements in management, yet, the rate of recurrence is still high reaching up to 15%–30% (17). Although using adjuvant treatment reduced recurrence rate but it till now has many complications (1). Thus, it is essential to discover novel biomarker for early prediction of recurrence which in succes-

**Table IV.** Association between patients treatment-response, outcome and expression of NR2F6, LOXL2 and DMBT1 in the cervical cancer patients.

	NR2F6			LOXL2			DMBT1		
	Low	High	p	Low	High	p	Low	High	p
	N = 28 (45.2%)	N = 34 (54.8%)		N = 28 (45.2%)	N = 34 (54.8%)		N = 40 (64.5%)	N = 22 (35.5%)	
<b>Treatment response:</b>									
CR	27 (96.4)	10 (29.4)		26 (92.9)	11 (32.4)		32 (57.5)	14 (63.6)	
PR	1 (3.6)	3 (8.8)	< 0.001*	2 (7.1)	2 (5.9)	< 0.001*	3 (7.5)	1 (4.5)	0.033
SD	0 (0)	7 (20.6)		0 (0)	7 (20.6)		4 (10)	3 (13.6)	
PD	0 (0)	14 (41.2)		0 (0)	14 (41.2)		10 (25)	4 (18.2)	
<b>Response:</b>									
OAR	28 (100)	13 (38.2)	< 0.001*	28 (100)	13 (38.2)	< 0.001*	26 (65)	15 (68.2)	0.033
NR	0 (0)	25 (61.8)		0 (0)	21 (61.8)		14 (35)	7 (31.8)	
<b>Recurrence (n=38):</b>									
Absent	16 (59.3)	1 (9.1)	< 0.001*	17 (63)	0 (0)	< 0.001*	7 (30.4)	10 (66.7)	0.028*
Present	11 (40.7)	10 (90.9)		10 (37)	11 (100)		16 (69.6)	5 (33.3)	

\*p < 0.05 is statistically significant; \*Chi square test.

sion will lead to discovering new targeted therapies against recurrence. We showed that NR2F6 expression was significantly associated with recurrence and it might be considered a predictive factor of recurrence and a cancer therapeutic target (18).

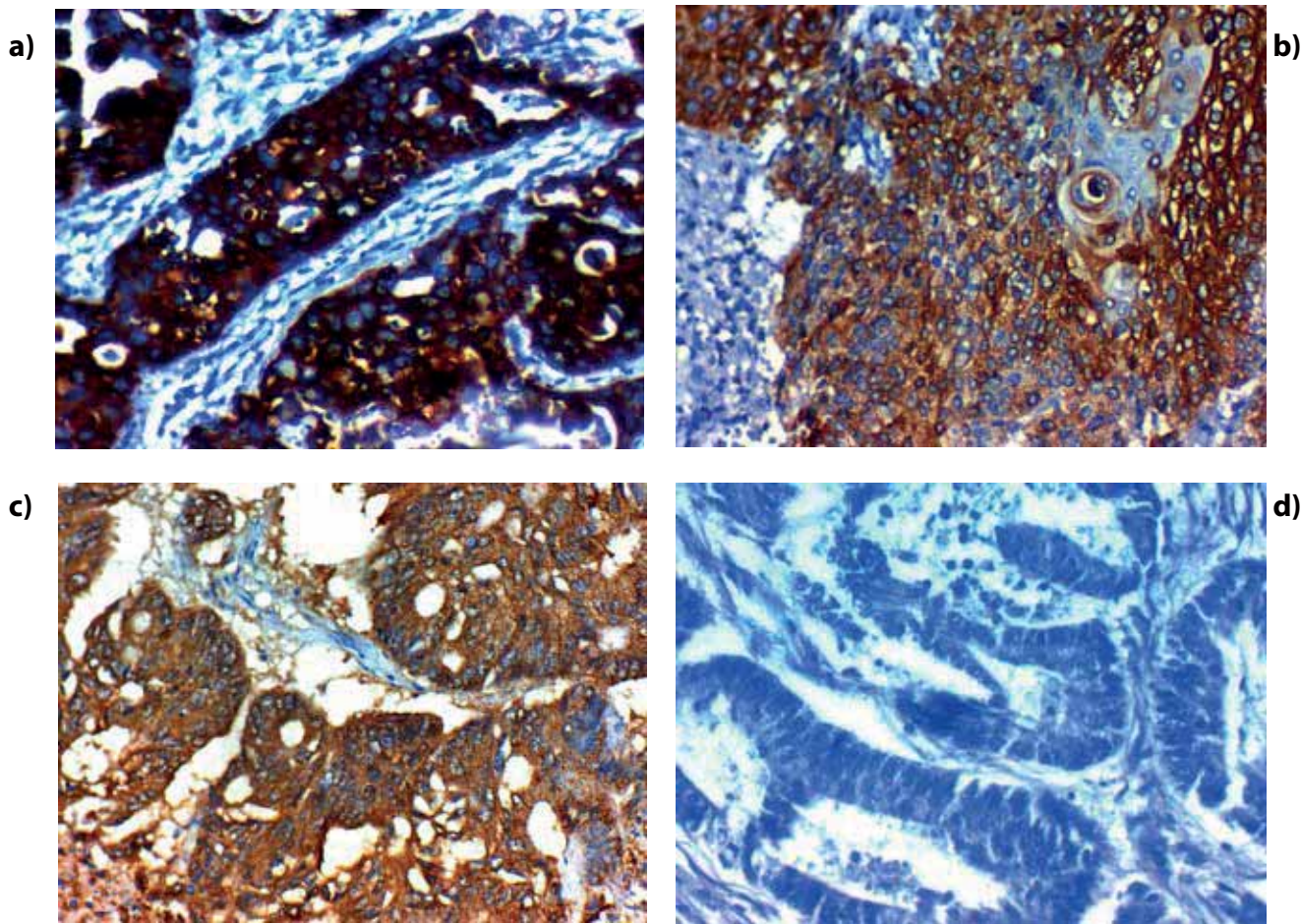
In the present study we assessed the expression of LOXL2 a novel cancer prognostic biomarker and we found that its expression was positively associated with NR2F6 expression and was related to unfavorable pathological and clinical findings, in addition, patients with high expression of LOXL-2 had poor survival rates.

We have chosen LOXL-2 in cervical cancer tissues due to previous studies about its association with

EMT induction in cancer through interaction with Snail-1 and E-cadherin (19).

Cao *et al.*, (10) found that LOXL2 expression was positively related to EMT phenotype in cancer cervix and they found that cancer cells proliferation and migration were reduced after inhibition of LOXL2. Cao *et al.*, (10) found similar results regarding association between high LOXL2 expression and poor OS and DFS in cancer cervix patients.

Aberrant activation of EMT in many types of cancer was found during tumor progression and metastasis (20). LOXL2 overexpression induced EMT by increasing expression of many EMT inducing factors as SNAIL, ZEB, FAK/SRC and IRE1-XBP1



**Figure 2.** LOXL2 expression in tissues of cervical carcinoma and non neoplastic cervical epithelium: (A) increased cytoplasmic expression in poorly differentiated invasive cervical squamous cell carcinoma Grade IIIx400, (B) increased cytoplasmic expression in moderately differentiated invasive cervical squamous cell carcinoma Grade IIx400, (C) increased cytoplasmic expression in moderately differentiated invasive cervical adeno-carcinoma Grade IIx400, (D) negative cytoplasmic expression in well differentiated cervical adeno-carcinoma of the cervix x400, (E) negative cytoplasmic expression in non neoplastic cervical epithelium of the ecto-cervix x400.

signaling pathways in cancer cells (21, 22). Additionally, LOXL2 has a role in remodeling process of the extracellular matrix which facilitated cancer cells invasion (23). Due to the scarcity of studies that assessed the expression of LOXL2 in cancer in addition to plenty of recent studies which were not significant particularly in regards to the grade and stage relation with its expression Cao *et al.*, (10), we assessed another marker DMBT1 expression and its association with NRF2 and LOXL2 in cancer cervix.

In the present report we showed that DMBT1 expression levels were down regulated in cervical cancer tissues and its low expression was associated with poor prognosis and dismal outcome. Similar results were found by previous studies (14). Zhang (14) showed that mRNA and tissue protein DMBT1 levels were downregulated in cervical cancer tissues in comparison to normal cervical mucosa. These findings collectively showed DMBT1 tumor suppressor role in cervical cancer.

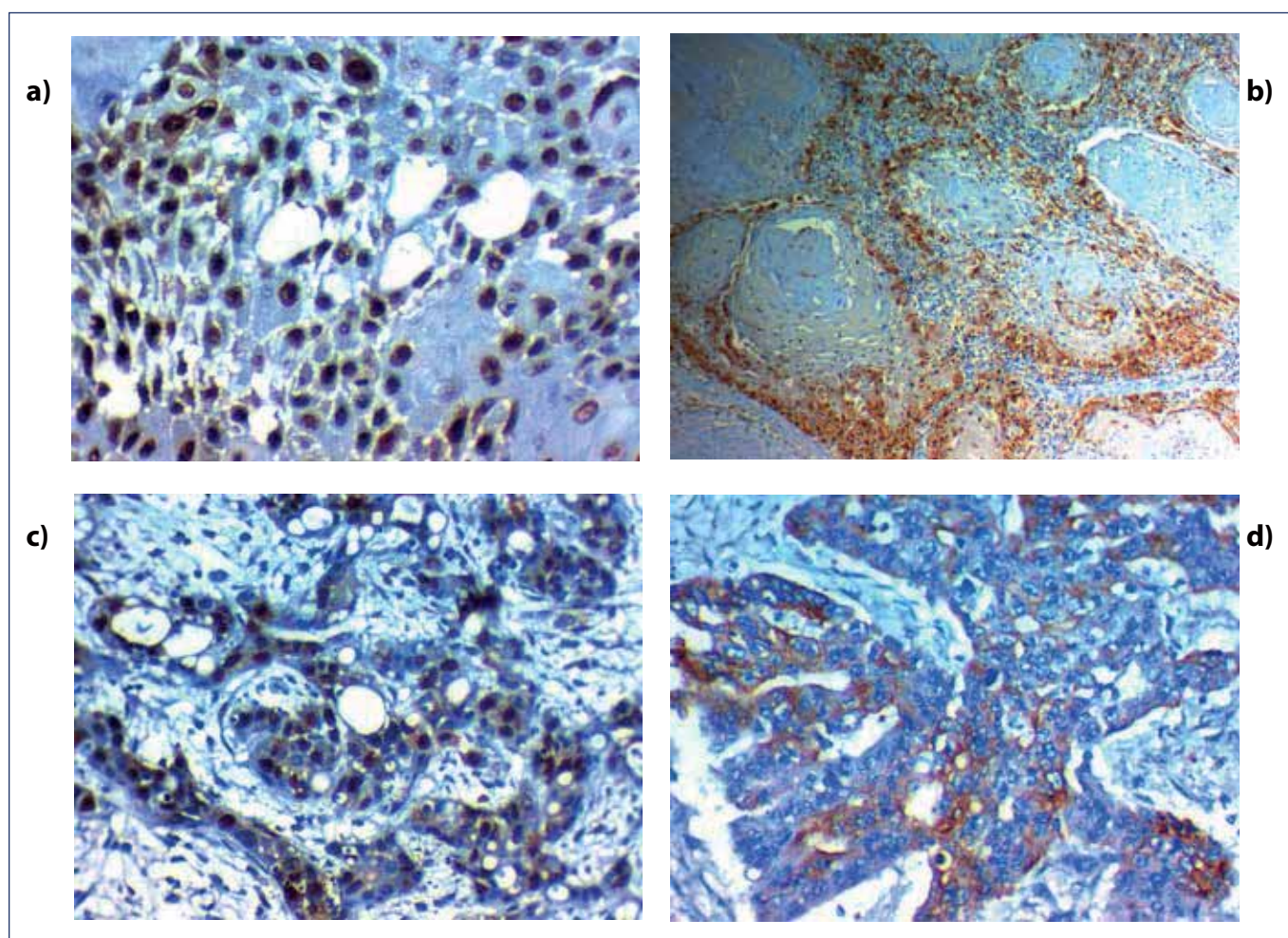
**Table V.** Association between patients survival and expression of NR2F6, LOXL2 and DMBT1 in the cervical cancer patients.

Patients' outcome	NR2F6		LOXL2		DMBT1	
	High	Low	High	Low	High	Low
	N = 34 (%)	N = 28 (%)	N = 34 (%)	N = 28 (%)	N = 22 (%)	N = 40 (%)
<b>Outcome:</b>						
Dead	23 (67.6)	2 (7.1)	24 (70.6)	1 (3.6)	18 (81.8)	19 (47.5)
Alive	11 (32.4)	26 (92.9)	10 (29.4)	27 (96.4)	4 (18.2)	21 (52.5)
P	< 0.001**		< 0.001**		< 0.008*	
Odds ratio	27.18		64.8		4.97	
95% confidence interval	5.45 – 136.68		7.72 – 544.14		1.43 – 17.34	

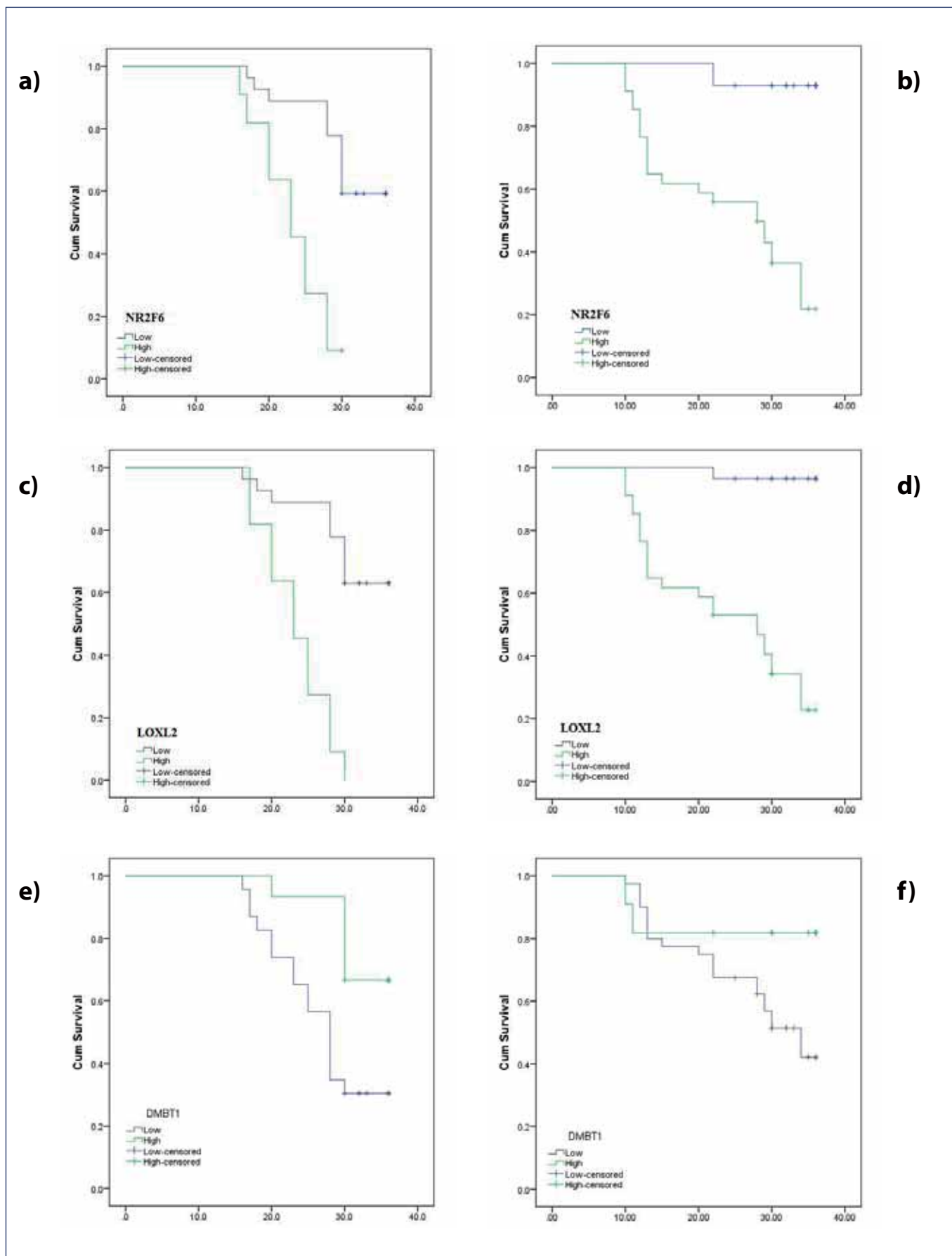
OR odds ratio; CI confidence interval; \*\*p ≤ 0.001 is statistically highly significant.

DMBT1 inhibition and association with cancer progression was found in many tumors (24). Additionally high expression of DMBT1 was associated with early FIGO stage, small tumor size, absence of lymph node metastasis, and low grade of the tumor. Sousa JF, (25) and Dodurga *et al.* (26) and Du *et al.* (27) agreed with our result and showed that low expression of DMBT1 was associated with advanced disease and poor prognosis of many cancers.

Loss of DMBT1 expression in colorectal cancer was associated with unfavorable prognosis and was a predictive factor for disease recurrence (28). Moreover, Zhang (14) found that overexpression of DMBT1 promotes apoptosis and inhibits proliferation of cervical cancer cells which might be a possible explanation of our results. Zhang (14) found both a marked elevation in the proapoptosis proteins as Bax and caspase-3 and



**Figure 3.** DMBT1 in tissues of cervical carcinoma and non neoplastic cervical epithelium: (A) High nuclear expression in non neoplastic cervical epithelium of the ecto-cervix x400, (B) High nuclear expression in well differentiated invasive squamous cell carcinoma of the cervix x400, (C) Low nuclear expression in poorly differentiated cervical squamous cell carcinoma x400, (D) Low nuclear expression in poorly differentiated cervical adeno-carcinoma x400.



**Figure 4.** Kaplan Meir survival curves of Disease free survival (DFS) and overall survival rate (OS) of cervical carcinoma patients: (A and B) DFS and OS rates stratified according to NR2F6 expression respectively, (C and D) DFS and OS rates stratified according to LOXL2 expression respectively, (E and F) DFS and OS rates stratified according to DMBT1 expression respectively.

a marked reduction in the expression of the anti-apoptosis protein (Bcl-2) in cancer cells with higher DMBT1 expression.

Moreover, they showed that overexpression of DMBT1 could inhibit cervical cancer cells invasion and metastasis.

It was found that disturbances in the PI3K-AKT signaling pathway was incriminated in EMT induction and in controlling cancer progression (29) Shen *et al.* (13) showed that higher expression of DMBT1 could be able to control PI3KAKT pathway by which it inhibits gall bladder cancer migration and invasion. Zhang *et al.*, (14) showed that increased DMBT1 expression led to reduction in the expression of Vimentin and N-cadherin and up-regulation of E-cadherin so DMBT1 was able to inhibit EMT reducing cancer cells invasion and spread.

## CONCLUSIONS

In the current study we assessed the expression of NR2F6, LOXL2 and DMBT1 which were novel markers incriminated in controlling EMT in cancer cells generally and particularly in cancer cervix cells. We concluded that up regulation of NR2F6, LOXL2 and down regulation of DMBT1 were associated with unfavorable pathological parameters, bad clinical findings and dismal outcome of patients.

### *Strengths of the study*

First, the study was a prospective study that was the first in evaluation of 3 novel biomarkers that have not been previously evaluated together. Second, the three markers were previously found to have similar roles regarding induction of EMT in variable cancers, but they were not sufficiently evaluated in cancer cervix.

Moreover, previous studies assessed each marker in squamous cell carcinoma (SCC) which is the commonest histopathological subtype of cancer cervix but we assessed the expression of the markers in both SCC and adenocarcinoma.

### *Limitations of the study*

First, the study included small cohort. Second, evaluation of the markers was done by only immunohistochemistry for evaluation of tissue protein expression of the assessed markers without genetic assessment.

So further future studies are needed to prove our findings in a larger cohort of cervical cancer patients and evaluation of genetic levels of the markers was needed to detect the mechanism of action of NR2F6, LOXL2 and DMBT1 in cervical cancer progression aiming at discovering novel therapies to decrease cervical cancer recurrence and progression.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## Postpartum readmissions and emergency department care following vaginal delivery in an Italian region

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### ABSTRACT

The objective of this study was to estimate hospital readmission and Emergency Department visit rates in puerperium among women discharged from the Italian Hospital of Udine after vaginal delivery. Administrative health databases of the Hospital were used as source of information. Readmissions or visits occurring within 42 days from deliveries recorded from 2000 to 2018 were analysed. Vaginal deliveries were 20756, postpartum readmissions 99 (0.48%) and ED visits 292 (1.41%). Readmissions occurred after a median time of 9 days from discharge, with median length of stay of 4 days. Postpartum ED visits occurred after a median of 15 days; 12.7% were yellow triage tags and 0.7% were red tags. Causes of readmissions and visits did not include only the specific complications of pregnancy, childbirth and puerperium; they differed in case of readmission or visit. Thus, readmissions only depict part of the postpartum hospital care needs of women after vaginal deliveries.

### SOMMARIO

L'obiettivo di questo studio era di stimare i tassi di riammissione ospedaliera e di visita nel puerperio al Pronto Soccorso tra le donne dimesse dall'Ospedale Italiano di Udine dopo il parto vaginale. Come fonte di informazioni sono state utilizzate le banche dati sanitarie amministrative dell'Ospedale. Sono state analizzate le riammissioni o le avvenute visite entro 42 giorni dai parti registrati dal 2000 al 2018. I parti vaginali sono stati 20756, le riammissioni postpartum 99 (0,48%) e le visite al pronto soccorso 292 (1,41%). Le riammissioni sono avvenute dopo un tempo mediano di 9 giorni dalla dimissione, con una permanenza media di 4 giorni. Le visite postpartum al pronto soccorso si sono verificate dopo una media di 15 giorni; il 12,7% erano codici triage gialli e lo 0,7% erano codici triage rossi. Le cause delle riammissioni e delle visite non includevano solo le complicanze specifiche della gravidanza, del parto e del puerperio, differivano in caso di riammissione o visita. Pertanto, le riammissioni rappresentano solo una parte delle esigenze di assistenza ospedaliera postpartum delle donne dopo il parto vaginale.

### IMPACT STATEMENT

#### *What is already known on this subject?*

Postpartum readmissions and Emergency Department treatment of urgent complications affect in particular women who had a caesarean section, however this issue also affects women after vaginal delivery.

#### *What the results of this study add?*

Almost 2% of women with a vaginal delivery at the University Hospital of Udine seek hospital care during puerperium: one fourth of them are readmitted whereas the majority is visited at the Emergency Department.

#### *What the implications are of these findings for clinical practice and/or further research?*

Problems requiring hospital care are not necessarily the same of patients only visited at the Emergency Department and thus require different solutions.

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### **Key words**

*Puerperium; hospital readmission; Emergency Department; administrative database; Italy.*

## **INTRODUCTION**

Great attention is given to hospital readmissions as a phenomenon related to quality and cost of health care. In Italy, they are monitored across different health conditions, including stroke, chronic obstructive pulmonary disease, joint replacement surgery, heart failure, and deliveries, within the national program of outcome evaluation Programma Nazionale Esiti (PNE) (1) which is run by the National Agency for the Regional Health Services Agenas through the analysis of a common set of variables included in hospital discharge data from all the Italian Regions. According to PNE, hospital readmissions during puerperium ranged between 0.7 and 0.9 per 100 after Caesarean delivery, and between 0.5 and 0.6 per 100 after natural delivery in Italian hospitals from 2009 to 2016 (1) with enormous variability across Regions and even hospitals, despite the indicators were risk adjusted to take into account the potentially confounding effect of the inhomogeneous distribution of maternal characteristics as recorded in the hospital discharge database. Little is known about the effect of other factors not recorded in such database. In addition, reasons for readmission have not been investigated.

In the United States, a study analysing deliveries in various States and including caesarean deliveries and multiple gestations, found that readmission rate raised from 1.72% in 2004 to 2.16% in 2011; more than half readmissions occurred within the first 10 days and the most common indications for readmission were hypertensive disorders, post-delivery infections, psychiatric diseases, and gallbladder disorders. The study identified predictors of postpartum readmissions, including income, maternal comorbidities, hospital characteristics, and pregnancy characteristics (*e.g.* caesarean delivery and multiple gestation) (2). However, despite the raising frequency of caesarean delivery, and the increased risk of readmissions in case of caesarean or

operative vaginal delivery (3), the increasing readmission rate in the United States appeared to be related more to maternal comorbidities than to mode of delivery (4). Other studies conducted all over the world confirmed higher readmission rate following caesarean deliveries than vaginal deliveries (5-8).

Hospital readmissions, however, are not the only type of hospital care for women in their postpartum. In fact, many urgent complications not resulting in a new hospitalization may be treated at the Emergency Department. In a Californian study, for example, ED visits within 90 days of delivery were analyzed: approximately 8% of women used the ED at least once, with half visits made in the first 3 weeks after discharge from the delivery hospitalization (9). The likelihood of an ED visit was higher among younger mothers and in those who had a caesarean delivery, had severe maternal morbidity at birth or pregnancy complications. A small association was also observed between the risk of ED visit and length of stay (9). The most common primary diagnoses were complications of puerperium, urinary tract infections, complications of obstetric surgical wounds, gallbladder calculus, haemorrhage, abdominal pain, and inflammatory disease of breast (9), only partially overlapping with discharge diagnoses reported in hospital readmissions (2).

The Italian University Hospital of Udine, with more than 1500 annual deliveries, is one of the largest birthing centres of the 1,200,000-inhabitant Italian North-eastern Region Friuli Venezia Giulia. According to PNE, readmission rates in Udine are not significantly different from the national average values, both for natural and for caesarean deliveries (1), and it is one of the best performing hospitals in the Friuli Venezia Giulia Region for these indicators. Nonetheless, the PNE indicators do not take into account other types of access to medical care in puerperium, such as ED visits, whose frequency is unknown.

This study has the objectives to estimate both hospital readmission and ED visit rates in puerperium

among the women who were discharged from the University Hospital of Udine after a vaginal delivery, to describe the causes of readmission or visit, and to investigate possible associations with characteristics of women, pregnancy, delivery, and newborns.

## MATERIALS AND METHODS

The health administrative databases of the University Hospital of Udine were used as the sources of information. In particular, the database of the population living in the Udine area, the hospital discharge database, the delivery certificate database, and the Emergency Department (ED) database were analysed. These databases are completely anonymous, but they can be linked deterministically at the individual patient level through a stochastic key which is univocal in all databases. The study period spans from 2000, which is the first year for which all the databases are available, and 2018, the most recent available year.

Hospital discharge records with a Diagnosis Related Groups (DRG) codes 372-375, corresponding to vaginal deliveries (372: vaginal delivery with complicating diagnoses; 373: vaginal delivery without complicating diagnoses; 374: vaginal delivery with sterilization and /or dilation and curettage; 375: vaginal delivery with operating room procedure except sterilization and/or dilation and curettage), were extracted from the Hospital of Udine database and linked with the corresponding delivery certificates, matched by individual stochastic key and date of delivery, which had to be included in the hospital stay period. Multiple deliveries according to the delivery certificates and cases with in-hospital maternal death were excluded from further analyses.

The overall hospital stay for the delivery hospitalization and the days from delivery to hospital discharge were analysed for each study year to assess possible trends.

Postpartum readmissions were identified as those hospitalizations occurring from the delivery hospital discharge to 42 days after delivery. Only urgent readmissions with length of stay > 1 day were considered. For women with no such readmissions, ED visits occurring in the same period were searched. The associations between readmissions or ED visits and characteristics of the women (age and educational level as reported in the delivery certificate; area of residency and citizenship from the population database, and Charlson's comorbidity index

in the previous 2 years calculated from hospital discharge data), of the father (age as reported in the delivery certificate), of the newborn (sex, birth weight, and 5-minutes Apgar score from the delivery certificate), of the pregnancy (overall number of obstetrical visits, use of assisted reproductive technology from delivery certificate), of the delivery (spontaneous or instrumental, days from delivery to discharge, complicating diagnoses in DRG) were assessed through chi-square tests for categorical variables and through t-tests for continuous numerical variables. P-values < 0.05 were considered statistically significant. Multivariate logistic regression analyses were also conducted to adjust for the potentially confounding effect of each variable on the others. The odds ratios (OR) and 95% confidence intervals (95%CI) were presented.

Details on readmissions (days after delivery discharge, hospital stay, main discharge diagnosis) and on ED visits (days after delivery discharge, cause of access, triage tag) were also described. All the analyses were conducted using SAS v9.4 (SAS Institute Inc., Cary, NC, USA).

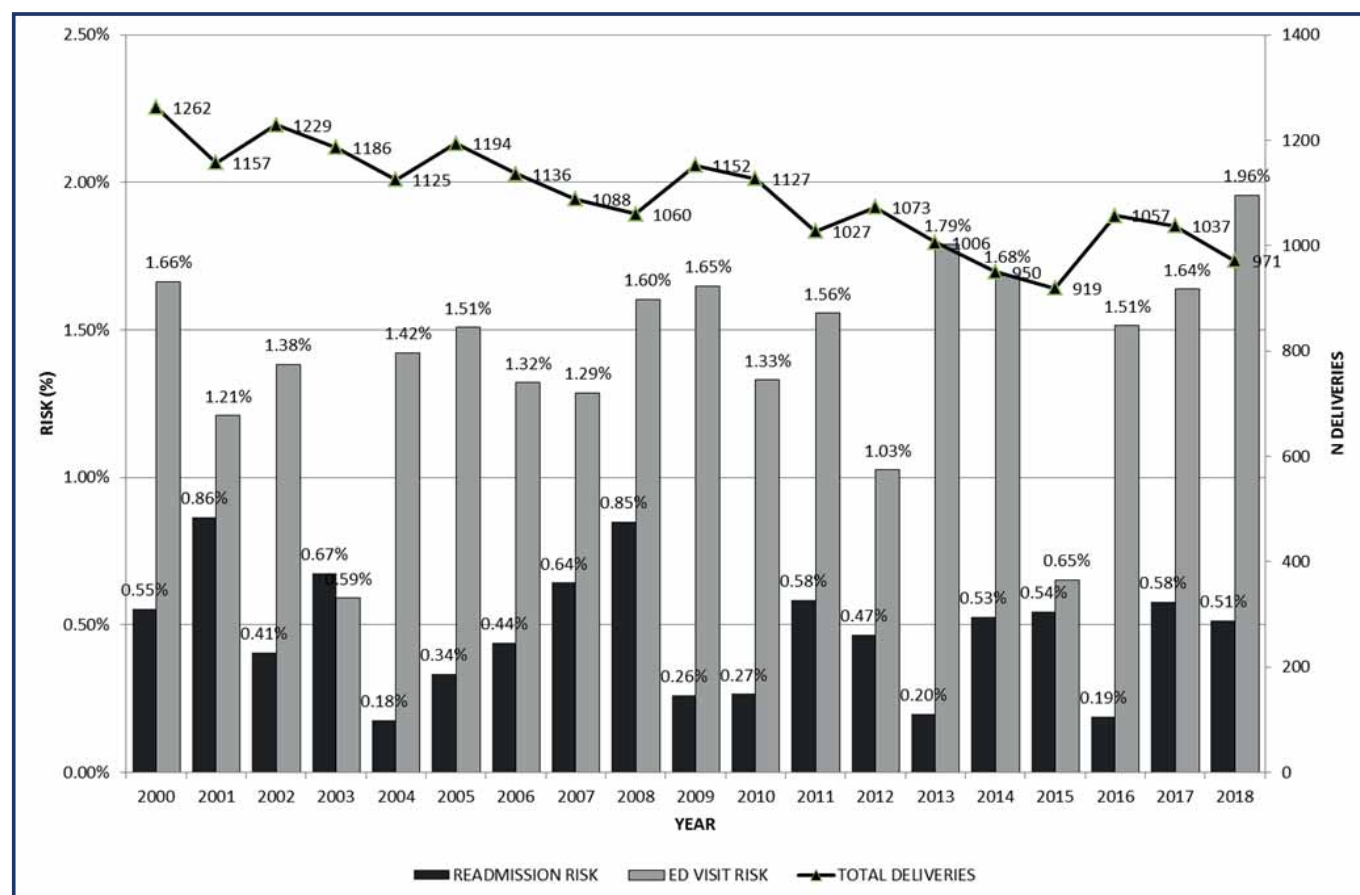
### *Compliance with ethical standards*

This work complies with the ethical standards of the relevant national and institutional committees on human experimentation and the Helsinki Declaration of 1975 as revised in 2008. This article does not contain any studies with of human or animal subjects performed by any of the authors. Since this analysis was based on anonymous administrative data, patient informed consent cannot be obtained and Ethical Committee approval was not required in Italy.

## RESULTS

Overall, 20756 deliveries were included in the analyses. Postpartum readmissions were 99 (0.48%) and ED visits 292 (1.41%). The annual distribution of readmissions and ED visits is shown in **figure 1**. The number of deliveries shows a decreasing trend; on the other hand, no clear pattern appears in the risk of postpartum readmissions and ED visits.

The median hospital stay of the delivery hospitalization was 4 days in all study years; the median of the days from delivery to hospital discharge was 3 days in all years but one (**table I**). However, the mean values of both measures showed a slightly



**Figure 1.** Annual number of vaginal deliveries at the University Hospital of Udine, Italy, and risk of postpartum readmissions and Emergency Department visits up to 42 days after delivery, years 2000–2018.

decreasing trend (p-value for linear trend < 0.0001 and 0.0068, respectively).

The associations between the categorical variables regarding characteristics of mothers, fathers, newborns, pregnancies, and deliveries and the risk of postpartum readmission or ED visit are illustrated in **table II**. Among variables measured as continuous numbers, no significant difference between readmitted and non-readmitted women or between women attending or not attending ED were observed according to the number of obstetrical visits during the pregnancy. On the other hand, readmitted women were on average slightly younger than the others (median age 31 *vs* 32, p of Wilcoxon's Rank Sums test 0.0941), as were those attending ED (median 31) as compared with the others (median 32; p-value < 0.0001). Analogous results were found for father's median age: 34 years in case of readmission *vs* 35 for no readmission (p-value 0.1618); 33 years in case of ED visit *vs* 35 for no ED visit (p-value 0.0007).

The results of logistic regression analyses assessing factor associated with postpartum readmissions and ED visits are shown in **table III**.

The average time from discharge from the delivery hospitalization and postpartum readmission was  $12.0 \pm 10.6$  days (median 9); average length of stay of postpartum readmissions was  $5.2 \pm 6.4$  days (median 4). Postpartum ED visits occurred on average  $16.4 \pm 11.3$  days after discharge (median 15 days). White triage tags, indicating non-urgent visits, were assigned to 128 cases (43.8%); 125 (42.8%) were green tags, 37 (12.7%) were yellow tags, and 2 (0.7%) were red tags indicating unstable patient conditions. 242 visits (82.9%) were due to medical non-accidental conditions. The diagnoses of the postpartum readmissions and ED visits are shown in **table IV**.

## DISCUSSION

This study showed that almost 2% of women who had a vaginal delivery at the University Hospital of Udine between 2000 and 2018 sought hospital care during puerperium: approximately one fourth of them were readmitted, whereas the others were only visited at the ED. Both the readmission and the ED visit rates had a fluctuating yearly pattern with

**Table I.** Delivery hospitalization length of stay and days from delivery to hospital discharge, University Hospital of Udine, Italy, 2000-2018.

Year	Length of stay		Days from delivery to hospital discharge	
	Mean ± standard deviation	Median	Mean ± standard deviation	Median
2000	4.03 ± 2.53	4	3.52 ± 1.04	3
2001	4.19 ± 2.42	4	3.65 ± 1.13	3
2002	4.19 ± 2.10	4	3.65 ± 1.17	3
2003	4.31 ± 3.01	4	3.69 ± 1.24	3
2004	4.37 ± 3.88	4	3.77 ± 1.07	3
2005	4.15 ± 1.89	4	3.60 ± 0.93	3
2006	4.16 ± 2.19	4	3.65 ± 0.87	3
2007	4.28 ± 3.96	4	3.59 ± 0.97	3
2008	4.25 ± 1.76	4	3.70 ± 0.93	4
2009	4.32 ± 3.05	4	3.66 ± 0.93	3
2010	4.08 ± 1.48	4	3.58 ± 0.92	3
2011	4.15 ± 2.33	4	3.66 ± 1.30	3
2012	4.11 ± 1.77	4	3.76 ± 6.09	3
2013	4.23 ± 3.26	4	3.49 ± 1.30	3
2014	4.09 ± 2.08	4	3.46 ± 0.93	3
2015	4.22 ± 3.33	4	3.38 ± 0.86	3
2016	4.06 ± 2.79	4	3.29 ± 0.88	3
2017	3.97 ± 2.90	4	3.30 ± 0.90	3
2018	3.97 ± 1.71	4	3.28 ± 1.07	3

no clear trend. On the other hand, the delivery hospitalization overall length of stay and the days from delivery to discharge showed a slightly decreasing trend, although the median stays were substantially unchanged during the observation period.

In this hospital, late discharges were associated with increased risk of both hospital readmissions and of ED visits in puerperium. One possible explanation is residual confounding from factors that determine both longer stays and subsequent complications requiring readmission. The issue of unmeasured confounding was raised also in a study of length of stay and readmissions among newborns (10). As an alternative, longer stays during the delivery hospitalization may increase the risk of nosocomial infections, as feared by others (11), although, looking at the causes of readmission, this hypothesis seems unlikely.

Mothers of premature newborns had increased risk of ED visit in puerperium. Both child prematurity and Apgar score < 7 were associated with increased risk of readmission, although non-significantly. The association between maternal comorbidity, as measured by the Charlson’s index, and use of post-partum hospital care was inconsistent and non-significant.

Women of non-Italian citizenship and those living out of the hospital catchment area were less likely to be visited at the ED. The last finding may, how-

ever, be an artefact, because the administrative data used for this study do not include information on hospital care received in hospitals other than Udine for non-resident subjects: women who delivered in Udine but lived elsewhere might have been subsequently treated at other hospitals. In case of serious reasons leading to readmission, it is likely that those women had returned to Udine, but for less serious conditions it is possible that they sought care at smaller peripheral EDs. To a lesser extent, the same limitation may affect the results about women living in the Udine area, whose readmissions or ED visits might have been missed if they occurred at hospitals not covered by the administrative databases used in these analyses.

The finding that mothers of female newborns were less likely to have ED visits in puerperium is unexplained. Another study showed that female newborns have been shown to have decreased risk of readmission themselves (10). In our study, however, the inconsistency between the direction of the association of the newborn’s sex with readmissions and with ED visits suggests that the association may be casual and not causal.

We expected to find a reduced likelihood of post-partum hospital use in case of spontaneous deliveries, as shown elsewhere (5). Although we observed such association it was not statistically significant in our population.

**Table II.** Associations between the characteristics of mothers, fathers, newborns, pregnancies, and deliveries (categorical variables) and the risk of postpartum readmission or ED visit, University Hospital of Udine, Italy, 2000-2018.

	Total	% readmitted	p-value	% ED visits	p-value
<b>Mother's educational level</b>			0.6676		0.0600
Elementary/middle school	6145	0.54		1.68	
High school	10306	0.47		1.36	
University degree	4305	0.42		1.14	
<b>Mother's citizenship</b>			0.1683		< 0.0001
Italian	16466	0.44		1.15	
Other	4290	0.61		2.38	
<b>Mother's residency out of area</b>			0.4283		0.0265
No	16084	0.50		1.50	
Yes	4672	0.41		1.07	
<b>Mother's Charlson's index</b>			0.4792		1.0000
0	20620	0.48		1.41	
≥ 1	136	0.74		0.74	
<b>Assisted reproductive technology</b>			0.3230		1.0000
No	20513	0.47		1.41	
Yes	243	0.82		1.23	
<b>Type of vaginal delivery</b>			0.1850		0.4629
Spontaneous	18474	0.45		1.39	
Instrumental	2282	0.66		1.58	
<b>Complicated delivery (DRG 372)</b>			0.7590		0.7839
No	20171	0.48		1.40	
Yes	585	0.51		1.54	
<b>Gestational age</b>			0.3754		0.0213
< 37 weeks	19487	0.65		2.21	
≥ 37 weeks	1085	0.46		1.37	
<b>Newborn's gender</b>			0.3421		0.0224
Female	10333	0.52		1.22	
Male	10423	0.43		1.59	
<b>Newborn's weight</b>			0.3710		0.7114
< 2500 g	882	0.79		1.70	
2500-4199 g	19294	0.46		1.39	
≥ 4200 g	580	0.52		1.55	
<b>5-minute Apgar</b>			0.0231		0.3842
≥ 7	20530	0.46		1.42	
< 7	226	1.77		0.44	

Among the most frequent causes of readmission, as expected, were postpartum haemorrhage and inflammatory disease of pelvic organs. Postpartum haemorrhage was the most common cause of readmission after vaginal delivery in Ireland (7), in Israel (6) and also in Australia, where it accounted for 18% of readmissions (8). In Ireland, atonic postpartum haemorrhage rates have shown an increasing time trend also among vaginal deliveries (12).

Among causes leading to ED visits but no subsequent hospitalization was thoracic or abdominal pain, which was also described as a cause of ED visit in the United States and of hospital readmission

in Australia, as were mastitis and infections of the breast (8, 9). Unspecified fever was a very common cause of both readmission and ED visit in our population.

Haemorrhoids were frequently reported as a cause of ED visit, as were thrombosis and embolism. Life threatening conditions such as acute myocardial infarction, cerebrovascular disease, heart failure were observed, despite being rare.

Psychoses were also a cause of ED visit, as they were in the United States (9), and even readmissions, as in Australia (8).

Gallbladder diseases, including cholelithiasis, were described among causes of both readmission

**Table III.** Adjusted measures of association between the characteristics of mothers, fathers, newborns, pregnancies, and deliveries and the risk of postpartum readmission or ED visit, University Hospital of Udine, Italy, 2000-2018.

	Readmissions		ED visits	
	OR (95%CI)	p-value	OR (95%CI)	p-value
Mother's age (years)	1.00 (0.95-1.05)	0.9931	0.98 (0.95-1.01)	0.2338
Father's age (years)	0.99 (0.96-1.02)	0.4514	0.99 (0.97-1.01)	0.5534
Mother's residency out of area	0.80 (0.48-1.34)	0.3966	0.71 (0.52-0.96)	0.0285
Mother's university degree (vs high school)	0.87 (0.50-1.53)	0.6379	0.95 (0.68-1.32)	0.7565
Mother's elementary/middle school (vs high school)	1.02 (0.64-1.62)	0.9300	1.14 (0.88-1.48)	0.3237
Mother's non-Italian citizenship	0.76 (0.45-1.28)	0.3082	0.56 (0.42-0.74)	< 0.0001
Mother's Charlson's comorbidity index $\geq 1$	1.64 (0.23-11.91)	0.6239	0.57 (0.08-4.01)	0.5747
Assisted reproductive technology	0.97 (0.13-7.10)	0.9756	1.03 (0.32-3.27)	0.9625
Number of obstetrical visits in pregnancy	1.07 (0.96-1.20)	0.2087	0.95 (0.90-1.01)	0.1018
Spontaneous delivery	0.69 (0.40-1.21)	0.2005	0.87 (0.61-1.24)	0.4337
Complicated delivery (DRG 372)	0.91 (0.29-2.92)	0.8808	1.02 (0.52-2.01)	0.9487
Gestational age < 37 weeks	1.19 (0.49-2.97)	0.7052	1.67 (1.02-2.74)	0.0421
Number of days from delivery to discharge	1.17 (1.08-1.28)	0.0002	1.08 (0.99-1.18)	0.0943
Female newborn's sex	1.30 (0.87-1.96)	0.2029	0.77 (0.61-0.97)	0.0291
Newborn's birth weight < 2500 g	1.17 (0.40-3.43)	0.7778	0.98 (0.51-1.87)	0.9409
Newborn's 5-minute Apgar score <7	2.71 (0.63-11.73)	0.1809	< 0.01 (0-inf)	0.9753
Year of delivery	0.97 (0.92-1.01)	0.1813	1.01 (0.99-1.04)	0.2819

sion and ED visit. This finding is consistent with reports from other studies (8, 9). In fact, impaired gallbladder motility in late pregnancy, with consequent increased risk of gallstone formation, has been known for long time (13).

In our population, the median time from delivery hospitalization discharge to readmission or ED visit was 12 and 16 days, respectively. In the United States, Batra *et al.* found that half ED visits occurred within the first 3 weeks (9). Readmissions lasted an average of 5 days, more than the delivery hospitalization itself. Among ED visits, almost half were assigned a white triage tag, meaning that they were actually due to non-urgent conditions. Women who have recently given birth to a baby may have post-discharge care needs which go beyond the classical postpartum complications and in fact several ED visits were prompted by non-specific symptoms and many conditions were actually non-urgent: better information of new mothers about the challenges that they might face during puerperium could help reducing inappropriate ED visits. Information on personal hygiene and appropriate post-partum lifestyle could also prevent some complications, such as mastitis or other genital infections. Other types of complications, *e.g.*, retained placenta or infections of surgical site, on the other hand, can be prevented by actions on the hospital side. For example, to reduce the risk of postpartum haem-

orrhage, regular emergency training focused on haemorrhage drills for the obstetric team, multidisciplinary teamwork, improved patient-team communication, and clinical audits have been advocated (12).

## CONCLUSIONS

In conclusion, this study showed that postpartum readmissions only depict part of the postpartum hospital care needs of women after vaginal deliveries, with the ED being largely utilized in the Italian Region Friuli Venezia Giulia. Only few maternal, pregnancy, and newborn factors were associated with increase postpartum hospital use. Problems requiring hospital care are not necessarily always the same in case of readmission or ED visit. Each problem should be addressed with appropriate interventions to prevent unnecessary postpartum hospital use, even in a birthing centre where, according to national performance evaluation systems, readmissions after vaginal delivery are not critical.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

**Table IV.** Groups of main discharge diagnoses of postpartum hospital readmissions and postpartum ED discharge diagnoses, University Hospital of Udine, Italy, 2000-2018.

Main diagnosis	Readmissions N (rate per 10,000 deliveries)	ED visits N (rate per 10,000 deliveries)
Non-urinary infections	-	5 (2.4)
Neoplasm	2 (1.0)	-
Blood disease	1 (0.5)	1 (0.5)
Psychoses and mental disorders	3 (1.4)	3 (1.4)
Disease of central nervous system	-	5 (2.4)
Diseases of peripheral nervous system and sense organs	-	12 (5.8)
Hypertension	4 (1.9)	3 (1.4)
Acute myocardial infarction	1 (0.5)	-
Acute heart failure	1 (0.5)	-
Dysrhythmia	-	1 (0.5)
Cerebrovascular disease	1 (0.5)	1 (0.5)
Thrombophlebitis, venous embolism and thrombosis	-	6 (2.9)
Hemorrhoids	1 (0.5)	8 (3.9)
Respiratory disease	-	7 (3.4)
Appendicitis	2 (1.0)	-
Cholelithiasis and gallbladder disease	5 (2.4)	5 (2.4)
Peritonitis	1 (0.5)	-
Acute necrosis of liver	1 (0.5)	-
Acute pancreatitis	1 (0.5)	-
Other diseases of digestive organs	2 (1.0)	14 (6.7)
Inflammatory disease of pelvic organs	8 (3.9)	14 (6.7)
Genitourinary and puerperal infection	5 (2.4)	-
Anemia	1 (0.5)	-
Postpartum hemorrhage	23 (11.1)	-
Retained placenta	8 (3.9)	-
Fever	11 (5.3)	7 (3.4)
Surgical complications	5 (2.4)	-
Mastitis and breast abscess	5 (2.4)	4 (1.9)
Other postpartum complications	1 (0.5)	1 (0.5)
Skin disease	-	11 (5.3)
Musculoskeletal disorder	-	7 (3.4)
Headache	1 (0.5)	4 (1.9)
Thoracic and abdominal pain	-	21 (10.1)
Other ill-defined signs and symptoms	-	45 (21.7)
Injury	-	13 (6.3)
Allergy	-	12 (5.8)
Other or non-specified	5 (2.4)	82 (39.5)

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## Obstetric nursing reorganization in pregnancy during the Covid-19 pandemic: a proposal integrative review of the literature

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### ABSTRACT

**Introduction.** Nursing management aimed to prevent viral infection in order to promote care plans that reduce the SARS-CoV-2 infection as much as possible.

**Evidence acquisition.** There were different studies focusing on small case series on Covid-19 pregnant women and on logistic management in order to reduce the risk of contagion.

**Evidence synthesis.** A review of the primary and the secondary literature was carried out in the Clinical Key and the PubMed databases. The bibliographic research was focused on the critical reading of the studies from 1 February 2020 to 30 June 2020. A total of eight articles analyzed special measures necessary to prevent Covid-19 in the pregnant women by implementing algorithms to be shared in a multidisciplinary context, also on challenges in the nursing care and in proposals for future studies.

**Conclusions.** Since there were few data available on the Covid-19 positive pregnant women, further developments of studies and more appropriate protocols were needed on this particular topic.

### SOMMARIO

**Introduzione.** La gestione infermieristica mira a prevenire l'infezione virale al fine di promuovere piani di cura che possano ridurre il più possibile l'infezione da SARS-CoV-2.

**Acquisizione di prove.** Ci sono stati diversi studi incentrati su piccole serie di casi su donne gestanti affette da Covid-19 e sulla loro gestione al fine di ridurre il rischio di contagio.

**Sintesi delle prove.** È stata effettuata una revisione della letteratura primaria e secondaria nei database Clinical Key e PubMed. La ricerca bibliografica è stata focalizzata sulla lettura critica degli studi dall'1 febbraio 2020 al 30 giugno 2020. Un totale di otto articoli hanno analizzato misure speciali necessarie per prevenire il Covid-19 nelle donne in gravidanza implementando algoritmi da condividere in un contesto multidisciplinare, anche sulle sfide nell'assistenza infermieristica e nelle proposte di studi futuri.

**Conclusioni.** Attualmente sono pochi gli studi disponibili sulle donne in gravidanza positive al Covid-19, pertanto ulteriori sviluppi di studi e di protocolli più appropriati su questo particolare argomento sono auspicabili.

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**Key words**

*Nursing-midwifery assistance; labor management; pregnancy; SARS-CoV-2 infection; birth.*

## INTRODUCTION

In late December 2019, a new coronavirus pneumonia (Covid-19) began to manifest itself in the Chinese city of Wuhan, and then spread nationally and internationally (1, 2). On 30 January 2020, the World Health Organization declared the global Covid-19 epidemic as an international public health emergency (3). In this scenario literature is unable to define the risk and the lethality index in Covid-19 general population (4), specifically in pregnant women (5). Already during past pandemic viral infections, as SARS in 2004 (6) and MERS in 2009, pregnant women were identified as more vulnerable than general population (7).

At this regard, in 2009, 5% of all H1N1-infection deaths regarded pregnant women and in other cases, SARS-CoV and MERS-CoV infections induced severe complications, such as endotracheal intubation, hospitalization in intensive care, impaired renal function for the pregnant patients. To date 56 pregnant women, Covid-19 positive, during the second and the third trimesters of pregnancy reported symptoms as: fever, cough, two thirds of patients recorded lymphocytopenia and the C-Reactive Protein increasing, and 83% of cases recorded multiple patches of ground-glass opacity in the lungs at the chest computer tomography (8).

Additionally, a case series of 12 Covid-19 pregnant women in Hong Kong, China, reported three maternal deaths; four of seven patients who presented in the first trimester had miscarriage; four of five patients had preterm birth; and two mothers recovered without delivery but their ongoing pregnancies were complicated by FGR (< 8) (9). Furthermore, it was not entirely clear whether and how pregnant transferred the infection to her fetus as all samples testing negative for the Covid-19 and suggesting that there was no evidence of vertical transmission in women who developed Covid-19 pneumonia in late pregnancy. As this regard, only two cases of neonatal Covid-19 infections reported an outcome of postnatal transmission (10). Another study analyzing 38 pregnancies demonstrated that there were no confirmed cases on vertical infection from mothers with Covid-19 to their fetuses (11).

For this reason, nursing management focused on promotion to act care plans to reduce the SARS-CoV-2 infection as much as possible. The present review aimed to analyze the current literature available in order to evidence:

1. any special measures necessary to prevent the SARS-CoV-2 infection in the pregnant woman;

2. how nursing care changed during the Covid-19 pandemic for pregnant women;

3. the management of pregnant women suffered from Covid-19 and further aspects to implement in future studies.

## MATERIALS AND METHODS

To answer the research questions, a proposal integrative literature review was carried out including systematic reviews, meta-analyzes and randomized clinical trials, opinion of experts, editorials, regarding nursing management in pregnancy during the Covid-19 pandemic. Clinical Keys and Pubmed databases were consulted, retroactively from 1 February 2020 to 30 June 2020 in order to better analyze the reorganization on nursing care for pregnant women during the Covid-19 pandemic and the effects on them and their children in the containment of the SARS-CoV-2 infection (**table I**).

**Table I.** The PICO instrument to literature review conduction.

<b>P = Patients/Population</b>	Pregnant women who give birth during the February 2020 - June 2020 period.
<b>I = Interventions</b>	Reorganization of nursing care for pregnancy women during the Covid-19 pandemic period.
<b>C = Comparison</b>	Comparison with periods preceding the pandemic.
<b>O = Outcome</b>	Effect on: <ul style="list-style-type: none"> <li>• women</li> <li>• children</li> <li>• the containment of the spread of the pandemic.</li> </ul>

Key words used as free terms combined with the Boolean AND operator were: Coronavirus, Pregnancy, Nursing Management in Pregnant Women and Intrapartum Care (**table II**).

**Table II.** Combination of keywords used with the Boolean AND operator.

Database	Search string	Results
PubMed 1	Coronavirus AND Pregnancy	650
PubMed 2	Coronavirus AND Nursing Management in Pregnant Women	175
PubMed 3	Coronavirus AND Intrapartum Care	5
PubMed 4	Coronavirus AND Obstetric Management in Pregnant Women	60

Inclusion criteria concerned:

- studies published in the Clinical Keys and PubMed databases;
- studies published from February 2020 to June 2020;
- articles available in the full-text version;

- review, clinical trial, observational and descriptive studies underlining the nursing care in the pregnant women during the Covid-19 pandemic;
- studies published in English language.

Exclusion criteria included:

- nursing studies which did not directly refer to the Covid-19 pandemic period;
- articles published in other languages, excluded English language;
- articles published before February 2020 (before the pandemic period).

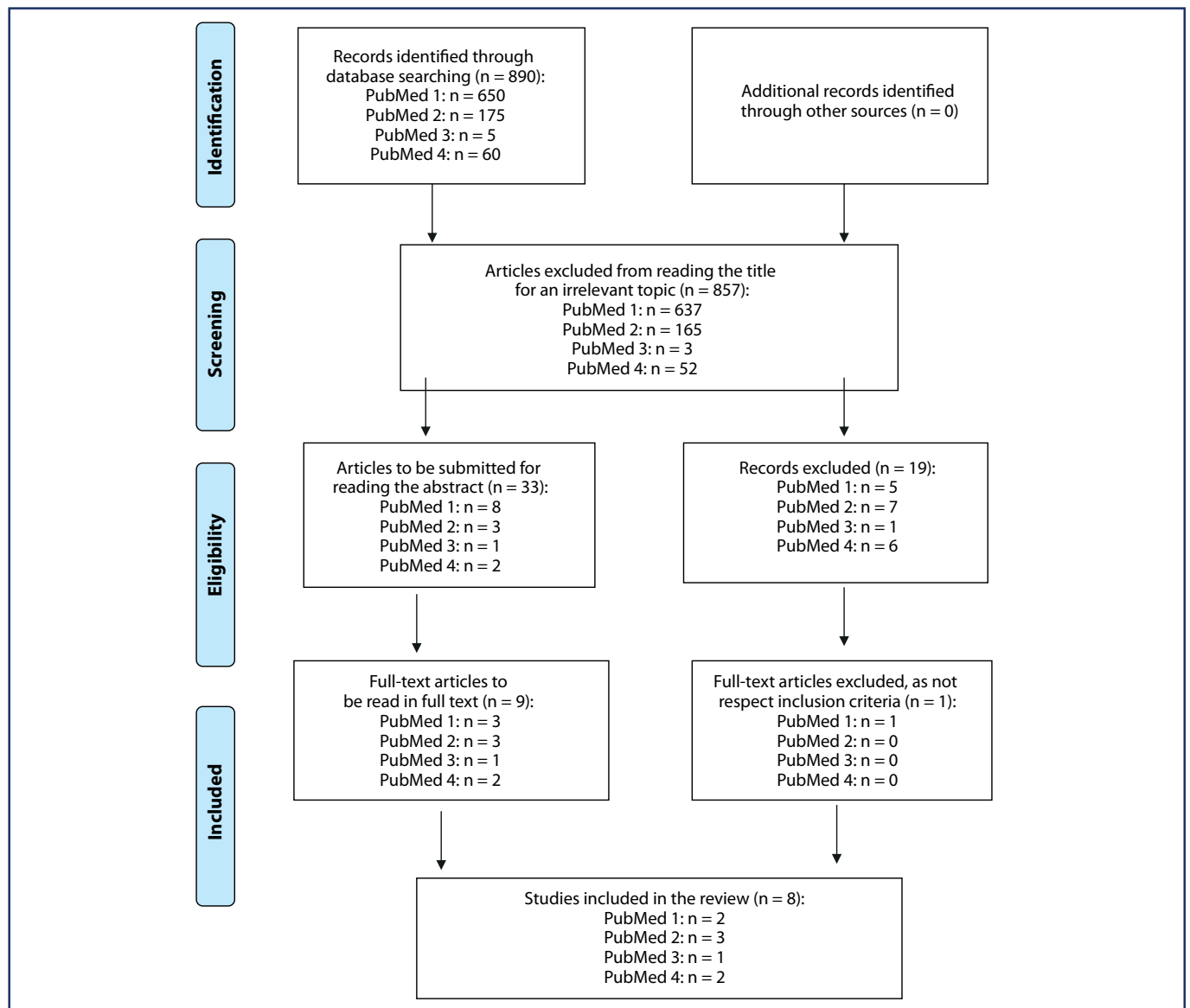
All studies recruited from this review were evaluated on the basis of: good description and appropriateness in study design (objective and method), sampling (sufficiently numerous, clarity of treatment allocation

criteria, absence of important bias), intervention, outcomes, statistic analysis and clinical relevance.

## RESULTS

The Clinical Key and the PubMed database searches provided a total of 890 studies for potential inclusion in the review (**figure 1**). After adjusting for duplicates and for an irrelevant topic in the title, 33 studies remained. Of these, 19 studies were discarded after reading and reviewing abstracts.

The full text of the remaining 9 studies was examined in greater detail. Of these, 1 study did not meet the inclusion criteria. Eight studies were finally included in this systematic review (12).



**Figure 1.** PRISMA 2009 Flow diagram for the proposal integrative review of the literature in: “Obstetric nursing reorganization in pregnancy during the Covid-19 pandemic”.

**Table III.** Special measures preventing SARS-CoV-2 infection in the pregnant woman.

Study Research Question/Statement	Findings	Reference
Maternal health care management strategies in woman who had fever and strategies including care planning and patient triage have been improving.	No nosocomial infection has occurred and none pregnant woman was reported to be infected: this management should be effective to an extent.	9
Could obstetric care provide safe and continuing essential services?	The clinical service model is improved in: workplace segregation; responsible social distancing, containment of cross-infection to healthcare providers; use of PPE; telemedicine.	13
An Italian regional model reorganization in the obstetric care.	The obstetric network was quickly reorganized with a model that guarantees assistance to all pregnant women identifying differentiated approaches according to the level of infectious and obstetric risk.	16

PPE: personal protective equipment.

Among the selected studies two were systematic reviews of the current literature (9, 13), one a retrospective multicenter case (14), two letters to Editor (15, 16), one case report (17), one a guidance (18) and one an opinion of experts (19).

Moreover, three studies (9, 13, 16) focused on the special measures necessary to prevent the SARS-CoV-2 infection in pregnant woman (**table III**), as: the implementation of maternal health care in patient triage to exclude any Covid-19 symptoms (9), or the improvement of work segregation, distancing, containment of cross-infection and the implementation of the personal protective equipment use (PPE) and the telemedicine practice (13). All these protective measures were well personalized to pregnant women in relation to both their level of infectious and their obstetric risk (16).

The other five studies (14, 15, 17-19) analyzed the main nursing care changes in pregnant women during the Covid-19 pandemic (**table IV**), by supporting innovative interventions in this context (14-15, 17-19), as: the earliest detection of severe illness and the individualized decisions on the adjunctive medications (14); the implementation of protocols in the use of PPE, as safety precaution for healthcare workers, the pregnancy management and its complications, including considerations to breastfeeding, mother to child infectious transmission, neonatal isolation devices (15, 17-19).

## DISCUSSION

### *Special measures necessary to prevent the SARS-CoV-2 infection in the pregnant woman*

To contain the Covid-19 pandemic a hospital network reorganization was proposed to share a guiding scheme for the entire obstetric hospital system in order to better manage pregnant women with

respect for providers and their own health (9, 13). The gynecology and obstetrics departments in the Italian territorial settings have been divided into Spoke and Hub centers. Specifically, the Spoke center guaranteed periodic prenatal care for pregnant Covid-19 women, while the Hub center performed examinations or a remote consultancy with a greater intensity of care (16). In this regard, a rearrangement in all these centers, both Spoke and Hub, was carried out both in the entry and the exit phase, as well as in the management of pregnant women who went to the laboratory for routine checks during pregnancy in order to minimize the risk of contagion (13, 16). Additionally, to avoid further spread of the epidemic, women were previously advised to stay at home and go to the hospital only in cases of strict necessity with a consequent dilemma for many pregnant women about whether to go to hospital (9), associated to a list of non-antiepидemic hospitals to consider and choose for a safer and a more regular obstetric consultation without complications and relative risk factors associated. Moreover, hospital rooms were reorganized as individually, to contain the SARS-CoV-2 infection with the inevitably consequent of a slowing admission rate. In addition, visitors were not allowed to access in the obstetric wards, except for particular cases in which only one person can be accepted. Also, an admission procedure was performed both for women with normal pregnancy and for urgent conditions, both including body temperature assessment and, if it exceeded 37.3 grades or there were particular respiratory signs, the anamnesis of the pregnant woman's contacts was performed to find the suspected Covid-19, including health professionals. In addition, shared protocols on triage were improved to direct the pregnant women to the right care setting and also, the improvement of the telemedicine approach was implemented, according to the principles of workplace segregation,

**Table IV.** Challenges in the nursing care for pregnant women during the COVID-19 pandemic.

Study Research Question/Statement	Findings	Reference
Critical care management of obstetrical patients with COVID-19 should generally be guided by the same principles as for the nonpregnant adult population?	The exploring treatment options include: early detection of severe illness and individualized decisions regarding the use of adjunctive medications.	14
Intrapartum care of women with COVID-19.	Management protocols for care providers, the proper utilization of PPE and scenario simulation, such as emergency cesarean section, should be improved in order to ensure efficiency in patient care as well as protective measures.	15
Experience of clinical management in pregnant women and newborns with COVID-19.	Management should include: inspection precautions; drug treatment options; indications and methods of termination of pregnancy; postpartum fever; breastfeeding considerations; mode of mother-to-child transmission; neonatal isolation and advice on neonatal nursing.	17
Recommendation for healthcare professionals on COVID-19 during pregnancy and puerperium.	Four algorithms to manage pregnancy in Covid-19 pandemic were performed: antenatal outpatient care; presentation to triage; intrapartum and postpartum management; neonatal care in women with suspected or confirmed Covid-19 infection.	18
Management of pregnant women infected with COVID-19.	More evidence is needed to establish which approach is safer to deliver and caesarean sections in this patient population.	19

FIGO: The International Federation of Gynecology and Obstetrics.

social distancing and containment of cross-infection (13). Finally, particular importance was given to the use of adequate PPE, with the organization of proper supply and distribution, along with appropriate training of all staff at risk of contagion (13). In all this complex context, a valid psychological support was requested for pregnant women and their families in order to reduce panic emotional conditions (9, 13, 16).

### *Challenges in the nursing care for pregnant women during the COVID-19 pandemic*

All the National Health Institutes published more updated treatment guidelines to contain the Covid-19 diffusion, including special considerations to pregnant women because, with their particular clinical condition, they were not embraced in the various clinical trials investigating treatment options for Covid-19 (15). Most guidelines published included: early detection of severe illness and individualized decisions on the care to be given to pregnant women; inspection precautions, drug treatment options, indications and methods of pregnancy termination, postpartum fever, breastfeeding considerations, mode of mother-to child transmission, neonatal isolation and advice on neonatal nursing in order to better manage the Covid-19 condition in pregnant women and their new born (17, 19).

The pregnant woman management with suspected or confirmed Covid-19 case was planned with a total of 4 algorithms, specifically: the antenatal outpatient care, the presentation to triage, the in-

trapartum and the postpartum management and the neonatal care in women with suspected or confirmed Covid-19 infection. Particularly, the key points for consideration in this plan were: the pregnant woman with an ascertained diagnosis of Covid-19 must be referred to a tertiary hospital and, at the same time, a detailed history regarding exposure relevant to Covid-19 and clinical manifestations should be collected. Suspected or confirmed cases must be treated in specialized hospitals with negative pressure isolation rooms also including neonatal units. In pregnant woman with fever or respiratory symptoms and the reduction in the total number of leukocytes, a swab test was performed and, if the mother was unable to take care of the new-born, he/she will be admitted to the neonatal ward remaining isolated until the successive swab test after 7 days. Finally, during the post-natal period, if the woman was Covid-19 positive and asymptomatic, she will be discharged 2 days after the birth and will be complied with the indications at home in isolation until at least 2 spaced 24 hours' negative tampons (16). Moreover, in occasion of suspected or confirmed cases, women should be quarantined immediately and they should be admitted in the specialized hospital for Covid-19 cases. Additionally, if the temperature reverted to normal for more than 3 days and the respiratory symptoms ameliorated, and the nucleic acid test for respiratory pathogens showed negative for two consecutive times, pregnant women could be discharged from the hospital or be transferred to the appropriate department.

Generally, literature supported that critical care management of obstetrical patients with Covid-19 should be guided by the same principles as for the non-pregnant adult population and was contingent on: the multidisciplinary approach, the close monitoring of maternal vital signs, the continuous fetal heart monitoring, the conservative fluid therapy and the oxygen therapy and, when it was necessary, the early neuraxial anesthesia with the elective instrumental delivery according to maternal condition and the stabilization of the critically ill patients (14, 15, 18, 19). Unique complication included decisions about fetal monitoring since prolonged administration of corticosteroids could in the long run caused lung maturity for fetal on short and therefore, the probability of early preterm delivery was high (15).

Moreover, in order to reduce cross-infection, healthcare institutes have improved the Internet consultation in order to fulfill online consultation and guidance for pregnant women (15, 20). Finally, all healthcare personnel must wear all the PPE necessary to contain the infection and should be trained for respirators (18). Furthermore, it would be desirable if the training already started from university nursing courses to perform trained nurses in the management of maxi emergencies (21, 22).

## CONCLUSIONS

### *Future perspectives*

Literature supported that due to the lack of experience they were uncertain of the clinical features, disease progress, outcome and treatment of pregnant Covid-19 patients, more work will be needed to fill in these gaps in the next future (5, 9). Most of the studies selected (14, 15) highlighted the limited availability data. So, clinically recommendations will surely continue to develop studies and more appropriate protocols evolving as regards microbiological, pharmacologic and clinical information about on Covid-19 in pregnant women, especially at the time of delivery. Currently, there was insufficient evidence regarding vertical mother-to-baby transmission in women affected by Covid-19 and regarding the safety of breastfeeding and the need for mother/baby separation (18, 20). Finally, the hope of all the works considered (5, 23) was to improve the available evidence on the condition of Covid-19

in the pregnant woman in order to implement the most appropriate care protocols (5, 23).

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## Effect of melatonin on postpartum hemorrhage in vaginal delivery: a prospective randomized double-blind study

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### ABSTRACT

**Background.** The similarities between the melatonin and oxytocin signaling could promote myometrium contractility. We conducted this study to determine the effect of melatonin on the bleeding during and after vaginal delivery.

**Methods.** The current double-blind randomized clinical trial was conducted on 140 pregnant women at term with labor pain. Subjects in the melatonin group received three sublingual doses of melatonin from labor room staff as follows: 6 mg in 7 cm dilatation, 3 mg after delivery of the fetus and 3 mg one hour after the delivery. The same schedule was conducted for the subjects in the placebo group by giving the placebo. The hemoglobin levels before and 24 hours after vaginal delivery, the hemodynamic variables were recorded.

**Results.** There was a significant difference between the groups regarding the mean of hematocrit changes in the melatonin ( $3.59 \pm 2.89$ ) and placebo ( $5.29 \pm 3.19$ ) groups ( $P = 0.001$ ). The mean variation of systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) show a significant difference among two groups of the study ( $P = 0.021$ ,  $P = .020$  and  $0.001$ , respectively).

**Conclusions.** The sublingual of melatonin to pregnant women with labor pain could reduce the amount of blood loss after the vaginal delivery. Furthermore, melatonin could provide hemodynamic stability.

### SOMMARIO

**Contesto.** Le somiglianze tra il signaling di melatonina e ossitocina potrebbero promuovere la contrattilità del miometrio. Abbiamo condotto questo studio per determinare l'effetto della melatonina sul sanguinamento durante e dopo il parto vaginale.

**Metodi.** L'attuale studio clinico randomizzato in doppio cieco è stato condotto su 140 donne in gravidanza a termine con dolore del travaglio. I soggetti nel gruppo della melatonina hanno ricevuto tre dosi sublinguali di melatonina dal personale della sala travaglio come segue: 6 mg con una dilatazione di 7 cm, 3 mg dopo il parto e 3 mg un'ora dopo il parto. Lo stesso programma è stato condotto per i soggetti nel gruppo placebo somministrando il placebo. Sono stati registrati i livelli di emoglobina prima e 24 ore dopo il parto vaginale e le variabili emodinamiche.

**Risultati.** C'era una differenza significativa tra i gruppi per quanto riguarda la media dei cambiamenti dell'ematocrito nei gruppi melatonina ( $3,59 \pm 2,89$ ) e placebo ( $5,29 \pm 3,19$ ) ( $P = 0,001$ ). La variazione media della pressione sanguigna sistolica (SBP), della pressione sanguigna diastolica (DBP) e della frequenza cardiaca (HR) mostra una differenza significativa tra i due gruppi dello studio ( $P = 0,021$ ,  $P = .020$  e  $0,001$ , rispettivamente).

**Conclusioni.** La somministrazione sublinguale di melatonina alle donne in gravidanza con dolore del travaglio potrebbe ridurre la quantità di perdita di sangue dopo il parto vaginale. Inoltre, la melatonina potrebbe fornire stabilità emodinamica.

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**Key words**

Melatonin; hemorrhage; vaginal delivery; postpartum hemorrhage.

## INTRODUCTION

Postpartum hemorrhages still one of the main causes of maternal death. Oxytocin is routinely prescribed by obstetricians to prevent and treat postpartum hemorrhages due to uterine atony after delivery. However, it may be insufficient for prevention of postpartum hemorrhage (PPH), for example in patients who have hepatorenal disease, psychotic disorders, chronic anemia, allergy to melatonin, long-term antidepressant or analgesic drug use. Oxytocin may cause severe cardio-vascular complications, such as hypotension, due to vasodilation, especially in high doses (1). Melatonin, N-acetyl-5-methoxy tryptamine, is a methoxyindole derived from tryptophan and totally secreted by hypophysis. Melatonin secreted rhythmically every day and stimulated by the suprachiasmatic nucleus of the hypothalamus. This hormone almost exists in all tissues (with or without receptor) of mammalian; this hormone acts as growth hormone and antioxidant (2). Melatonin can regulate circadian rhythm; it is sedative, pain reliever and has anti-inflammatory and anti-oxidant effects which encourage obstetricians to prescribe it (2). It is hypothesized that melatonin and oxytocin could cause augmenting the overnight contractions of term uterus. In other words, melatonin synergizes with oxytocin to promote contractility of human myometrial smooth muscle cells (3, 4). It is reported that melatonin levels increase in maternal blood, amniotic fluid and urine of pregnant women throughout pregnancy, reaching a peak at term (5). The beneficial effect of melatonin in placental and fetal well-being is reported (5-9). Melatonin especially causes the circadian rhythms and helps fetal growth and neurogenesis, while no adverse fetal or neonatal outcomes have been reported. Furthermore, it is shown melatonin repairs the injury with regrowth of axons and possesses neonatal cerebral protection and it could induce the condition to re-

establish periventricular white matter which may be associated with the enhancement of learning ability (5-9). In previous study, it is shown premedication of patients with 6 mg sublingual melatonin statistically reduced the amount of blood loss after lower segment cesarean section, which may not be clinically meaningful (10).

The use of melatonin as an anxiolytic agent has been studied in several humans and animals (2, 11), but to the best of our knowledge, this study is the first in which the effect of melatonin has been estimated on the amount of blood loss after vaginal delivery. Hence, the current randomly controlled clinical trial aimed to evaluate the effect of melatonin on the amount of bleeding during and after vaginal delivery.

## METHODS

Following Ethics Committee approval and informed patients' consent, 154 pregnant females whose age ranged between 18-40 years with American Society of Anesthesiologists (ASA) physical status of I or II who had been referred to the hospital due to spontaneous labor pain and were the candidates for vaginal delivery were recruited in a prospective, double-blinded randomized controlled trial in 2014-2015. The patients were randomly allocated to one of the two groups to receive either three sublingual doses of melatonin from labor room staff as follows: 6 mg in 7 cm dilatation, 3 mg after delivery of the fetus and 3 mg one hour after the delivery. The same schedule was conducted for the subjects in the control group by giving the placebo. Exclusion criteria were significant coexisting conditions such as hepatorenal disease, psychotic disorders, chronic anemia (hemoglobin Hb < 10 g/dl) cardiovascular disease, allergy to melatonin, long-term antidepressant or analgesic drug use, any risk factor associated

with an increased risk of postpartum hemorrhage, labor dystocia, distended uterus (macrosomia and multiple births), chorioamniotite and parity > 4, administration of uterine relaxants (halogenated anesthetic agents, beta-adrenergic agonist (sympathomimetic) and MgSO<sub>4</sub>), those who were under the induction of labor and patients who needed blood transfusions (after receiving the required blood) excluded from the study. Also, subjects who needed other drugs besides oxytocin to control postpartum bleeding, those who undergone cesarean or even needed blood transfusions were excluded from the study. Consolidated Standards of Reporting Trials (CONSORT) recommendations for reporting randomized, controlled clinical trials were charted (figure 1). The randomization was commenced using computer-generated random numbers in the closed opaque envelopes. The allocation was achieved by a resident physician who was out of the project and the drugs given by a nurse non-involved in the study. The obstetrician was blinded to the patient's group allocation, and a blinded observer recorded the study data. According to the routine protocol in the delivery section of our Hospital, all of patients

received intravenous infusion of oxytocin 20 units of oxytocin in 1 L of ringer lactate with speed of 1000 CC/h immediately after clamping the umbilical cord. The main outcome measures were the estimated of blood loss at vaginal delivery and change in hemoglobin levels. Since the difference of Hb provides a more accurate assessment of actual blood loss during vaginal delivery, than visual estimation, we determined the hemoglobin value before and 24 h following the vaginal delivery. Apgar score and hemodynamic variables after delivery were recorded. Sample size was calculated based on the change in hemoglobin level before (Hb1) and 24 h after vaginal delivery (Hb2) in a pilot sample of 20 patients. Considering 90% power and 5% error, the sample size was determined to be 60 cases in each group. We included 70 patients in each group to allow for dropouts and protocol violations. Data were analyzed using SPSS (SPSS 15.0, SPSS Inc., Chicago, IL, USA). Continuous variables were tested for normal distribution by the Kolmogorov–Smirnov test. Student's t test and paired t test were used for variables with normal distribution. Chi-square test was used to compare qualitative variables between

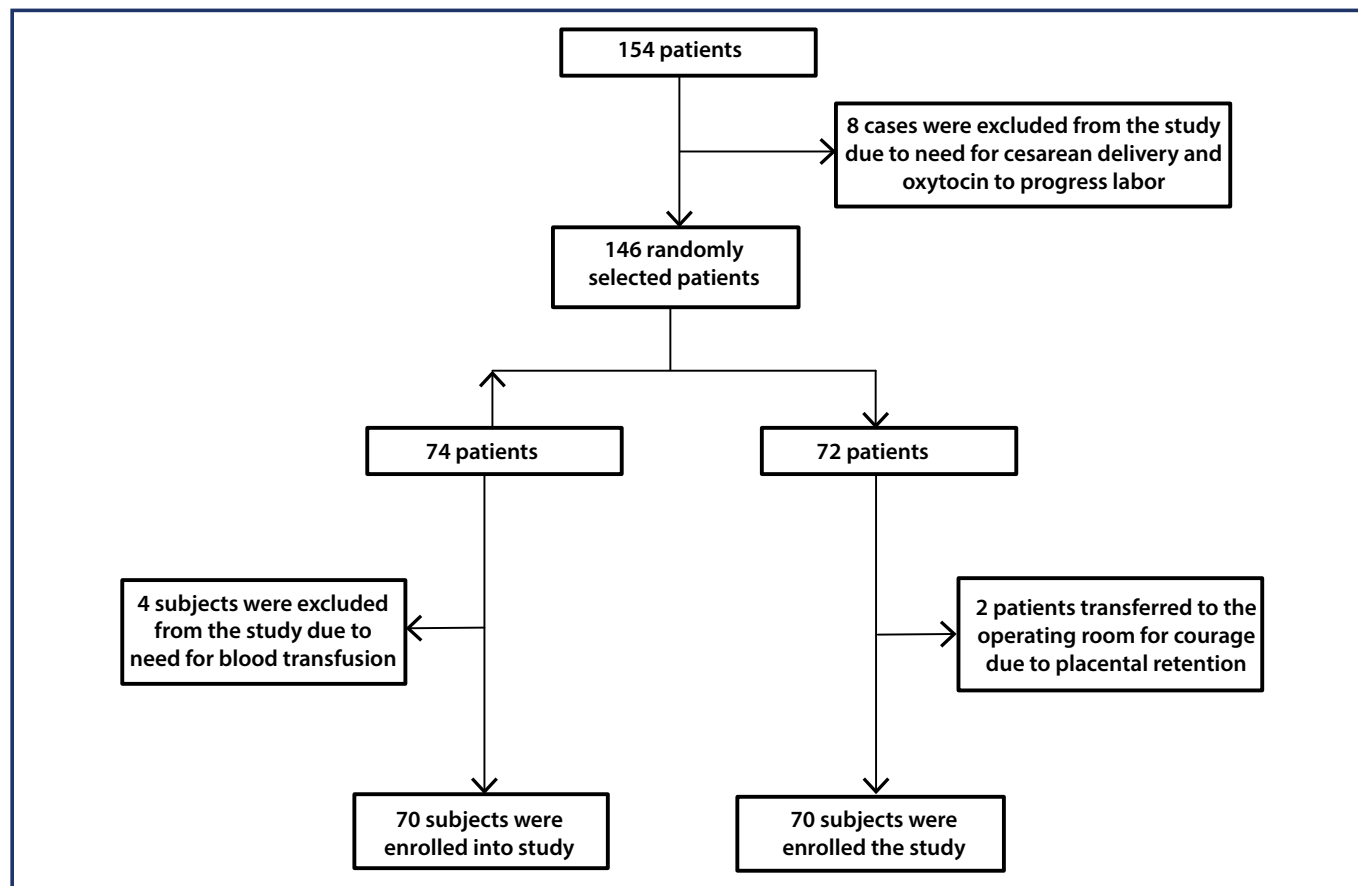


Figure 1. Consort Flow diagram.

groups. The P values of less than 0.05 were considered significant.

## RESULTS

One hundred and fifty-four patients were recruited in the study of which 14 were excluded due to logistic reasons or other factors violating the study protocol (figure 1). Table I shows the mean age of the subjects in the intervention and the control groups was  $26.19 \pm 4.72$  and  $25.41 \pm 4.74$  years old, respectively (table I). As shown in table I, no significant difference was observed between the groups regarding the level of hemoglobin during the time of labor; while there was a significant difference in this regard after labor between the groups ( $11.53 \pm 1.19$  g/dL in the intervention and  $11.05 \pm 1.32$  g/dL in the control groups) ( $P = 0.002$ ). The mean variations of Systolic Blood Pressure (SBP), Diastolic Blood Pressure (DBP) and heart rate (HR) were defined as the difference between the highest and the SBP, DBP and heart rate in each patient and compared between the groups. Table II shows that difference of SBP ( $P = 0.028$ ) and DBP ( $P = 0.022$ ) variation between two groups were significant. Also, as shown in table II the difference of mean HR variation between two groups were significant ( $P < 0.001$ ). All new borns in our study were free of any adverse effect. The Apgar scores at first ( $P = 0.891$ ) and five

( $P = 0.576$ ) minutes after delivery in the two groups were statistically similar.

## DISCUSSION

Based on the data found in our study, we concluded that sublingual administration of melatonin to pregnant women with spontaneous labor pain could reduce the amount of blood loss after the vaginal delivery, although it may not be clinically meaningful. The estimation of blood loss during vaginal delivery remains a challenge. The measuring blood loss during the third stage of labor by visual estimation is inaccurate. The effectiveness of quantitative blood loss measurement on clinical outcomes has not been demonstrated. A more precise measurement of blood loss is hemoglobin concentration (Hb) in venous blood sampling. The difference of Hb provides a more accurate assessment of actual blood loss than visual estimation. However, to avoid of bias in this research, patients who needed blood transfusions, additional uterotonic agents, or surgical interventions were considered as exclusion factors. The results of present study partially are consistent with the results of previous study by Khezri *et al.*, which conducted on patients undergoing cesarean section under spinal anesthesia (10). However, there were no differences regarding the hemodynamic conditions between the groups in the study by Khezri *et al.* The current study reported more stable hemodynamic conditions regarding the systolic and diastolic blood pressure ranges in the intervention group, in such a way that the changes were lower in the intervention group compared with those of the control group. The more stable hemodynamic variables in the current study in the melatonin group are a valuable finding. However, these effects may be dose-dependent. In

Table I. Comparing the main outcomes between the Groups.

Report	Group		P value
	Melatonin (n = 70)	Placebo (n = 70)	
	Mean ± SD	Mean ± SD	
Age (year)	26.19 ± 4.72	25.41 ± 4.74	.337
GA (week)	39.06 ± 1.04	39.04 ± 0.08	.928
Hemoglobin in labor (g/dl)	12.80 ± 1.04	12.65 ± 1.08	.417
Hemoglobin 24 h after delivery (mg/dl)	11.53 ± 1.19	11.05 ± 1.32	.026
HCT in labor (%)	37.56 ± 2.81	37.36 ± 2.67	.676
HCT after delivery (%)	33.96 ± 3.55	32.06 ± 3.70	.002
Change in HCT%	3.5929	5.2971	.001
Change in Hemoglobin (mg/dl)	1.2743	1.6057	.055
apgar1	8.79 ± 0.58	8.77 ± 0.64	.891
apgar5	9.91 ± 0.28	9.89 ± 0.32	.576
Placental removal (min)	2.08 ± .47	2.08 ± .47	1

Values are presented as mean ± SD.

Table II. Changes in hemodynamic variables.

	Group		P value
	Melatonin	placebo	
Change Systolic blood pressure (mmHg)	4.1429	7.0000	.028
Change Diastolic blood pressure (mmHg)	2.1429	5.0000	.022
Change Heart rate (bpm)	1.4000	- 3.5143	.000

Values are presented as mean ± SD; SBP; systolic blood pressure (mmHg); DBP: diastolic blood pressure; HR; heart rate (bpm). The mean variation of MAP and HR was defined as the difference between the highest and the lowest mean arterial pressure and heart rate in each patient.

current study, we use the higher dose of melatonin compared to Khezri *et al.* study (10). This finding is consistence with the result obtained in the Ismail's (2) and Gupta's (12) studies in which a significant decrease in mean arterial pressure (MAP), after melatonin premedication, was reported. This effect may be related to the modulatory effect of melatonin on the cardiovascular function. Moreover, the hemodynamic effect of melatonin may be attributed to its anxiolytic actions. The underlying mechanism is probably the synergy between melatonergic and GABAergic systems. It also has analgesic effects as observed by various investigators and this may also contribute to the hemodynamic stability (2, 12).

According to the studies by Sharkey *et al.* (3) and Kumari *et al.* (4), the similar structures of melatonin and oxytocin receptors in vitro showed that free calcium increase in platelets after exposure to melatonin. Considering the role of calcium to control bleeding and the role of melatonin on increasing the amount of intracellular free calcium in platelets, it can be concluded that calcium-mediated melatonin can decrease bleeding (4). Calcium also plays a role in deformation, adhesion and aggregation of platelets (4, 13-15). Finally, calcium, in physiological concentrations, can improve platelet aggregations. Therefore, it can be concluded that the possible role of melatonin on blood restriction results from increasing the hemostatic activities in platelet aggregations (4, 13-15). Accordingly, changes in the level of hematocrit in the intervention group were lower than those of the control group, and hemostatic processes in the current study were performed more effective and finally led to decrease of bleeding. Furthermore, hemodynamic effect of melatonin can be considered as one of the explanation causes of bleeding reduction in the current study. The current study is the first trial which showed the effectiveness of melatonin on decreasing the level of bleeding in vaginal delivery. However, it is far too early to reach any definitive conclusions. Future studies are necessary to evaluate the effect of melatonin on the level of bleeding in vaginal delivery.

## CONCLUSIONS

According to the results of the current study, it can be concluded that the sublingual administration of melatonin to pregnant women with spontaneous labor pain could reduce the amount of blood loss after the vaginal delivery. Furthermore, melatonin

could provide hemodynamic stability. So, using melatonin, as a supplementary agent in vaginal deliveries is explainable due to favorable hemostatic and hemodynamic effects.

## CONTRIBUTORS

The first author of this paper is Ezatossadat Haj SeyedJavadi, Associate Professor of Qazvin University of Medical Science, which responsible for conception and design of study. Mahor Kamali, Resident of Obstetrics and Gynecology as done acquisition of data and drafting the manuscript. Ameneh Barikani, Associate Professor of Community and preventive medicine who analyzed and interpreted the data and reviewed the manuscript. Professor Marzieh Beigom Khezri, Professor of Qazvin University of Medical Science, is the corresponding authors of this paper which responsible for conception and revising the manuscript critically for important intellectual content. Fatemeh Lalooha, Associate Professor of Qazvin University of Medical Sciences who cooperated in drafting and reviewing the manuscript.

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## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## Prevalence of low antithrombin levels in preeclamptic women and perinatal outcome

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### ABSTRACT

**Objective.** The aim of this study is to evaluate the prevalence of low antithrombin levels in our population in order to assess an intervention trial feasibility.

**Methods.** This is a retrospective study. A database was created by using queries to find out medical records of patients requiring hospitalization for preeclampsia or gestational hypertension or superimposed preeclampsia to chronic hypertension at Modena University Hospital between June 2015 and July 2019.

**Results.** We screened 11845 deliveries. Overall, 221 (1.9%) cases of preeclampsia were identified. Antithrombin level was available for 201 women, thus included in the analysis. Median antithrombin value was 87% (IQ range: 77-98). The prevalence of low antithrombin levels was 9%. Antithrombin < 80% was found in 21% of the subjects. The remnant showed normal values. Median antithrombin was significantly lower in severe respect with mild preeclampsia ( $83\% \pm 14$  vs  $89\% \pm 14$ ,  $p = 0.003$ ). The rate of small for gestational age was significantly higher in low antithrombin levels group ( $44.4\%$  vs  $22.4\%$ ,  $p = 0.042$ ). Considering mean values, antithrombin levels were also significantly lower in case of small for gestational age ( $84\% \pm 14$  vs  $89\% \pm 14$ ;  $p = 0.040$ ).

**Conclusions.** In our population, low antithrombin levels (1 in 10 patients) were associated with severity of preeclampsia, namely with small for gestational age babies. Data suggest this subpopulation as a better target for trials assessing the efficacy of antithrombin supplementation.

### SOMMARIO

**Obiettivo.** Lo scopo di questo studio è valutare la prevalenza di bassi livelli di antitrombina nella popolazione da noi studiata con lo scopo di valutare un trial di intervento.

**Metodi.** Si tratta di uno studio retrospettivo. È stato creato un database attingendo i dati dalle cartelle cliniche delle pazienti che sono state ricoverate per preeclampsia semplice o sovrainposta, ipertensione gestazionale presso l'Azienda Ospedaliero Universitaria di Modena dal giugno 2015 al luglio 2019.

**Risultati.** Sono state analizzate 11845 cartelle cliniche. Sono stati identificati 221 casi di preeclampsia (1.9%), i livelli di antitrombina erano stati dosati in 201 casi con un valore mediano di 87% (intervallo QI:77-98). La prevalenza di bassi livelli di antitrombina era del 9%. Livelli di antitrombina inferiori a 80% sono stati riscontrati nel 21% dei casi, mentre i restanti presentavano valori normali. I valori di antitrombina mediana erano significativamente più bassi in caso di preeclampsia grave ( $83\% \pm 14$  vs  $89\% \pm 14$ ;  $p < 0.003$ ).

La percentuale di neonati piccoli per l'età gestazionale era significativamente più alta nel gruppo con bassi livelli di antitrombina ( $44.4\%$  vs  $22.4\%$ ,  $p < 0,042$ ) e i valori medi di antitrombina erano più bassi in queste pazienti ( $84\% \pm 14$  vs  $89 \pm 14$ ;  $p < 0,003$ ).

**Conclusioni.** Nella nostra popolazione bassi livelli di antitrombina (1 su 10 pazienti) erano associati alla gravità della preeclampsia, in particolare se complicata dalla nascita di neonati piccoli per età gestazionale. I dati suggeriscono che questa popolazione rappresenta il miglior target per studi che vadano a valutare l'utilizzo di antitrombina.

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### **Key words**

*Antithrombin; gestational hypertension; preeclampsia; small for gestational age; chronic hypertension.*

## **INTRODUCTION**

Preeclampsia (PE) is a multifactorial disease, characterized by abnormal vascular response to placentation that is associated with increased systemic vascular resistance, enhanced platelet aggregation, activation of the coagulation system and endothelial dysfunction (1). The main pathological feature is the incomplete transformation of the spiral arteries, leading to hypoperfusion of the placenta and insufficient nutrient supplies to the fetus, and resulting in impaired vascular function and fetal growth restriction. Endothelial dysfunction is involved in the pathogenesis of preeclampsia. Endothelial damage triggers a thrombotic microangiopathy with platelets and coagulation factors consumption (2).

From a clinical point of view, PE is classified in two distinct subtypes based on the timing of onset: early-onset (< 34 weeks) and late-onset preeclampsia ( $\geq$  34 weeks). In a population-based study the overall preeclampsia rate was 3.1% with an incidence of early-onset and late-onset preeclampsia respectively of 0.38% and 2.72%. Early-onset preeclampsia confers a higher risk of maternal complications and perinatal mortality (3). Antithrombin (AT) is a glycoprotein involved in both coagulation system and inflammatory processes. AT is the principal inhibitor of thrombin and factor Xa, involved in both extrinsic and intrinsic coagulation pathways. When AT binds thrombin formed the irreversible thrombin-antithrombin (TAT) complex. Moreover, AT binds heparin-like glycosaminoglycans on the surface of endothelial cells, and promotes endothelial cell release of prostacyclin, which impacts inflammatory processes (4).

AT normally decreases during the third trimester of pregnancy and falls during postpartum period. In early-onset preeclampsia there are significantly reduced antithrombin levels, probably reflecting increased consumption, impaired liver function and urinary loss (5, 6).

In a previous observational prospective study, we reported that in patients with PE, a progressive reduction of AT values is associated with deteriorating clinical conditions leading to indication to delivery (7). Since AT reduction is a result of increased consumption due to the hypercoagulable state, and decreased hepatic synthesis (8), it has been speculated that AT administration could restore the AT levels. Therefore, clinical trials have been designed to assess if the AT administration in women with severe PE was associated with prolongation of pregnancy, improvement in fetal growth and maternal hypertension (9-12).

Our aim is thus to evaluate the prevalence of low AT levels in our population in order to assess an intervention trial feasibility.

## **METHODS**

This is a retrospective study and database was created by using queries to find out medical records of patients requiring hospitalization for PE or gestational hypertension or superimposed PE to chronic hypertension at Modena University Hospital which served as Hub for the Modena area. Each patient's file has been sorted and checked for consistency by two of us. Patients with diagnoses that falls within the ISSHP criteria (13) have been included. PE occurring at less than 34 weeks of gestation was identified as early-onset disease, whereas PE that occurred at 34 weeks or later was labeled late-onset disease. Severe PE was defined by blood pressure higher than 160/110 mmHg or signs and symptoms of organ involvement. Low AT levels were considered for AT < 70%.

Blood samples were collected immediately before birth in cases of expedite delivery till 2 days before for women already admitted to the Hospital. Patients received routine treatment with antenatal corticosteroids.

For every subject we collected information on age, ethnicity, body mass index, obstetric history, need of assisted reproductive technology (ART), small for gestational age (SGA) defined as birthweight  $\leq 10^{\text{th}}$  centile (corrected for gender and gestational age) diagnosis, gestational age at admission, gestational age at delivery, mode of delivery, AT levels before delivery, blood chemistry and coagulation profile. Outcomes of pregnancy were also collected. Statistical analysis was performed by using SPSS® Statistics software, version 19. Continuous variables were evaluated with Student t-test while categorical variables with chi-square test. AT levels distribution was analyzed as median and interquartile range, and comparison between groups was evaluated with U Mann-Whitney Test. A logistic regression analysis was performed to evaluate possible factors associated with low AT levels. Statistical significance was considered for p value less than 0.05.

## RESULTS

We screened 11845 deliveries occurring between June 2015 and July 2019. Overall, 221 (1.9%) cases of preeclampsia were identified. AT level in blood samples was available for 201 women which were included in the analysis.

Sociodemographic characteristics are described in **table I**. According to AT, there were no differences except for the higher rate of chronic hypertension among patients with normal AT levels.

We found no differences between normal AT group and low AT group in gestational age (GA) at admission as well as for other clinical features such as systolic and diastolic blood pressure at admission, rate of early onset PE or severe PE (**table II**).

Median AT value was 87% (IQ range: 77-98). Overall, the prevalence of low AT levels, considering AT  $< 70\%$  as a threshold, was 9%. AT  $< 80\%$  was found in 21% of the subjects. Seventy percent of women had normal AT levels (AT  $\geq 80\%$ ). Other laboratory tests did not differ between normal AT and low AT group, except for higher LDH levels in low AT group (**table III**).

Median AT values were not different in early onset PE (86%  $\pm 13$ ) vs late onset PE (88%  $\pm 14$ ) and no significant differences were found in AT  $< 70\%$  levels between early onset (7.4%) and late onset PE (9.5%). Considering the severity of clinical PE, median AT values were significantly lower in severe PE respect with mild PE (83%  $\pm 14$  vs 89%  $\pm 14$ ,  $p = 0.003$ ), but the rate of AT  $< 70\%$  levels did not differ (13.7% vs 7.3%). HELLP syndrome occurred in 6/201 cases (3.0%), 2 of them (33.3%) had AT  $< 70\%$ . AT levels were slightly, although not significantly, lower in patients with HELLP syndrome (78%  $\pm 20$  vs 88%  $\pm 14$ ).

**Table I.** Sociodemographic characteristics.

	Whole sample	AT $\geq 70\%$	AT $< 70\%$	p
	n = 201	n = 183 (91.0)	n = 18 (9.0)	
Age $\geq 35$	102 (50.7)	93 (50.8)	9 (50.0)	NS
Ethnicity				
Caucasian	138 (67.7)	126 (68.9)	10 (55.6)	NS
Sub-saharian	38 (18.9)	35 (19.1)	3 (16.7)	
Maghreb	12 (6.0)	9 (4.9)	3 (16.7)	
Others	15 (7.5)	13 (7.1)	2 (11.1)	
Low education ( $< 8$ yrs)	69 (34.3)	66 (36.1)	3 (16.7)	NS
Smoking during pregnancy	8 (4.0)	8 (4.4)	0	NS
Assisted reproductive technology	20 (10.0)	17 (9.3)	3 (16.7)	NS
Nulliparity	102 (50.7)	92 (50.3)	10 (55.6)	NS
Pregestational diabetes mellitus	6 (3.0)	5 (2.7)	1 (5.6)	NS
Gestational diabetes mellitus	57 (28.4)	51 (27.9)	6 (33.3)	NS
Preeclampsia in previous pregnancies	17 (8.5)	17 (9.3)	0	NS
Chronic hypertension	79 (39.3)	77 (42.1)	2 (11.1)	0.007
Pregestational BMI $\geq 30$	71 (35.3)	67 (36.8)	4 (22.2)	NS
Excessive weight gain	61 (30.3)	58 (31.7)	3 (16.7)	NS

**Table II.** Clinical characteristics.

	Whole sample	AT ≥ 70%	AT < 70%	p
	n = 201	n = 183 (91.0)	n = 18 (9.0)	
Gestational age at admission (days)	252 ± 29	251 ± 30	256 ± 18	NS
Systolic blood pressure at admission	148 ± 18	148 ± 19	152 ± 16	NS
Diastolic blood pressure at admission	91 ± 12	91 ± 12	94 ± 13	NS
Early onset preeclampsia	54 (26.9)	50 (27.3)	4 (22.2)	NS
Severe preeclampsia	51 (25.4)	44 (24.0)	7 (38.9)	NS

**Table III.** Laboratory tests.

	Whole sample	AT ≥ 70%	AT < 70%	p
	n = 201	n = 183 (91.0)	n = 18 (9.0)	
Antithrombin (mean ± SD)	87 ± 14	90 ± 12	63 ± 7	< 0.001
Antithrombin (median and IQ range)	87 (77-98)	89 (81-98)	66 (63.5-68)	< 0.001
Fibrinogen (mg/dl)	510 ± 95	513 ± 92	485 ± 120	NS
Hemoglobin (g/dl)	11.7 ± 1.3	11.7 ± 1.2	11.7 ± 1.5	NS
Hematocrit (%)	35 ± 4	35 ± 4	35 ± 4	NS
Platelets (103/mm <sup>3</sup> )	222 ± 67	224 ± 67	198 ± 64	NS
Serum Glutamic Pyruvic Transaminase (U/L)	32 ± 51	33 ± 53	24 ± 9	NS
Lactate dehydrogenase (U/L)	349 ± 96	342 ± 94	421 ± 82	0.001
Urine protein single sample (mg/dl)	91 ± 139	81 ± 130	184 ± 188	NS
Proteinuria (mg/24h)	2553 ± 3375	2603 ± 3456	1997 ± 2485	NS

**Table IV.** Maternal and fetal outcome.

	Whole sample	AT ≥ 70%	AT < 70%	p
	n = 201	n = 183 (91.0)	n = 18 (9.0)	
<b>Maternal</b>				
GA at delivery (days)	255 ± 26	255 ± 27	258 ± 17	NS
Birth < 37 weeks	77 (38.3)	69 (37.7)	8 (44.4)	NS
Latency (days)	2 (0-4)	2 (0-4)	1.5 (0-4)	NS
IOL	93 (46.3)	84 (45.9)	9 (50.0)	NS
Emergency CS	74 (36.8)	67 (36.6)	7 (38.9)	NS
<b>Neonatal</b>				
IUGR	69 (34.3)	59 (32.3)	10 (55.6)	0.045
SGA	49 (24.4)	41 (22.4)	8 (44.4)	0.042
IUFD	4 (2.0)	4 (2.2)	0	NS
NICU admission	55 (27.4)	47 (25.7)	8 (44.4)	NS

Maternal and neonatal outcomes are reported in **table IV**. GA at delivery was similar between groups. The mean latency from admission to delivery was 2 days (IQ range: 0-4 days), and it was similar in the two groups. Overall, 46.3% of women underwent an induction of labor. The rate of emergency cesarean section was 36.8% in the whole sample. The number of small for gestational age (SGA) babies was higher in low AT levels group (44.4% vs

22.4%,  $p = 0.042$ ) and also considering mean values, AT levels were significantly lower in case of SGA ( $84\% \pm 14$  vs  $89\% \pm 14$ ;  $p = 0.040$ ) respect with babies with appropriate birthweight.

In a logistic regression model including elevated maternal age (> 35 years), obesity (BMI > 30 kg/m<sup>2</sup>), pre-pregnancy hypertension (chronic hypertension), onset and severity of PE, only gestational hypertension seems to be associated with lower

AT levels ( $p = 0.029$ , R2-Nagelkerke 0.117, likelihood ratio 110.112).

## DISCUSSION

Low AT levels have been observed in PE in different studies, but there is no consensus on which threshold utilize. This make difficult to define the prevalence of reduced AT levels in such specific population. In our study the majority of subjects had normal value while low AT levels were found in about 9.0% of the women. Another study used the same threshold, although not focused on the detection of AT levels as primary outcome. They reported a prevalence of low AT levels in PE women which was nearly double (14) than here reported. Their sample size however was small and the number of primiparous women was higher than in our population.

Other studies have reported lower AT values (as a mean) in PE women respect than in our population. In such cases, however, only women with gestational hypertension were included (7, 15). A further study including only patients with gestational hypertension reported mean AT levels more similar to the one here reported (16).

As already reported, we found that low AT levels are more prevalent in women with severe PE (17). The same trend, however, was not confirmed in women with early onset PE. There were no other clinical features associated with low AT levels except in the cases of superimposed PE which seem protected again this deficit.

On these grounds, some Authors evaluated the effects of AT administration in preeclamptic women. In severe PE the administration of intravenous AT once a day, for seven days improved maternal symptoms, defined as gestosis index as well as fetal well being evaluated through the biophysical profile (10). In a small study involving women with early onset PE and IUGR fetuses the administration of both AT and heparin compared with heparin alone showed a decrease in systolic blood pressure and improved fetal growth in the treatment group (11). Another trial carried out in early onset severe PE demonstrated a preservation of fetal biophysical status and a prolongation of pregnancy in women treated with AT (12). Therefore, it seems AT supplementation could provide benefit for mothers and fetus, at least in japanese population. In an Italian randomized trial, an increase of AT levels was observed in treatment group, al-

though no differences were found in term of clinical outcomes due to small sample size. It has to be pointed out that the study was stopped by the sponsor because of the slow rate of enrolment (18). It is known that a hypercoagulability state is associated with the occurrence of small babies and this is counteracted by an increased formation of thrombin-antithrombin (TAT) complex (16, 19). These findings agree with the significantly decreased AT activity we found in cases of SGA.

## CONCLUSIONS

In conclusion, we reported that low AT levels occur in a significant proportion of women with PE, namely in those with severe features. Of paramount importance, SGA is strongly associated with reduced AT therefore supporting the implementation of large clinical trials evaluating the effects of AT supplementation.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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## Are we training too many doctors, or too few? The challenge of the increased residency slots

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To the Editor,

In Italy, after graduation in Medical School, a physician may opt for a residency training program or become a general practitioner. In the last 10 years, residency slots have been insufficient compared to the number of new graduated medical doctors, creating an imbalance between supply and demand for residency training programs. In this way, access to residency have become increasingly difficult. Also, the increased slots for access to the Medical School course, has amplified the phenomenon of the training funnel, that is the difference between the number of admissions to the degree course and the number of residency slots.

It is important to remember that the "limited slots" available for the Medical School course has a dual purpose: 1) to ensure the quality of the training based on settings and facilities and 2) to guarantee employment after graduation. On the other hand, any change in the number of admissions to the Medical School degree leads to a consequences in 10-12

years. Given the number of available residency slots each year, has been estimated that in the 2024 there will be over 20,000 graduated doctors without the possibility to get into a residency training program (**figure 1**).

There is no doubt that the training funnel creates serious generational damage with important implications for the doctor's professionalization. Furthermore, it should be emphasized that the shortage of medical staff in hospital wards and local services risks further abrupt acceleration with the introduction of the new pension rules, *i.e.*, "Quota 100". Physicians employed by the NHS, today retire with an average seniority of about 65 years old. In 2018, began the exit from the system of those born in 1953 (about 7,000 doctors). In the three-year period 2019-2021 (which will essentially affect those born between 1954 and 1956), are expected between 6,000 and 7,000 doctors to leave each year, for a total of about 20,000 units.

As found in the ANAAO study (1), about half (52,500) of the approximately 105,000 medical spe-

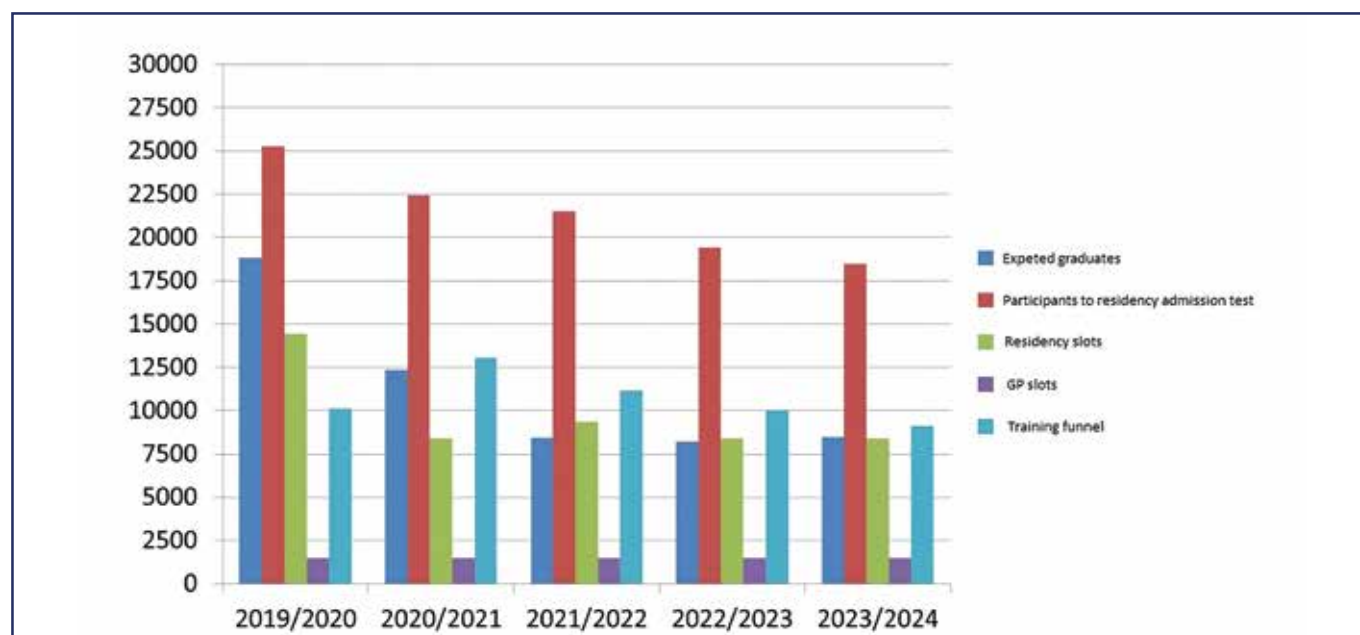


Figure 1. Training funnel between 2020 and 2024.

Adapted from ANAAO study. Available from <http://www.quotidianosanita.it/allegati/allegato893679.pdf>. Accessed 03/05/2021.

cialists currently employed in public health, will retire between 2018 and 2025.

For the above reasons in recent years the government has progressively increased the number of residency slots. Regarding *Obstetrics and Gynecology Residency Training Program*, in the 2020, there has been 463 slots available. In the 2015 only 239 slots were available. It means almost 100% increase in 5 years. During this period, almost none of the residency training programs (or in any case the vast minority) carried out a rescheduling of the training plan or substantial changes to the regulations to cope with this “revolution”. We believe that most schools are not at all equipped to cope with this increase and this situation is destined to worsen in the coming years, if no action is taken (2). Moreover, we need to face the challenge during a pandemic. A recent study showed that among Italian residents in obstetrics and gynecology, COVID-19 pandemic was associated with a significant training impairment (3). We do see a reduction in the number of hours that physicians and trainee work, generally, over time for both male and female physicians, that can have an effect on training.

We discussed this issue with all representatives of the obgyn trainees, within the AGUI (AGUI Specializzandi), and we recommend the following:

- increase the possibility of training in external facilities, also increasing the current 18-month threshold;
- improve the possibility of exchanges for training between the different residency training programs;

- wiser use of training networks, also by increasing the available locations;
- use of family counseling centers;
- use of the common trunk;
- change in rotations based on the trainees number;
- possible increase in the number of incall shifts (*i.e.*, 12/24 hours shifts) based on the number of trainees.

In summary, a national doctor planning model is urgently needed. We can take advance from the pandemic to face the problem of the training in the obgyn residency training program.

## CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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