

# Letrozole combined with norethisterone acetate compared with norethisterone acetate alone in the treatment of pain symptoms caused by endometriosis

S. Ferrero<sup>1,5</sup>, G. Camerini<sup>2</sup>, R. Seracchioli<sup>3</sup>, N. Ragni<sup>1</sup>, P.L. Venturini<sup>4</sup>, and V. Remorgida<sup>1</sup>

<sup>1</sup>Department of Obstetrics and Gynaecology, San Martino Hospital and University of Genoa, Largo R. Benzi 1 16132, Genoa, Italy

<sup>2</sup>Department of Surgery, San Martino Hospital and University of Genoa, Largo R. Benzi 1 16132, Genoa, Italy <sup>3</sup>Minimally Invasive Gynaecological Surgery Unit, Reproductive Medicine Unit, S. Orsola-Malpighi Hospital, University of Bologna 40138, Bologna, Italy

<sup>4</sup>Department of Obstetrics and Gynaecology, Institute G. Gaslini 16147, Genoa, Italy

<sup>5</sup>Correspondence address. Tel/Fax: +39 010511525; E-mail: dr@simoneferrero.com

**BACKGROUND:** The available data on effectiveness of aromatase inhibitors in treating pain symptoms related to endometriosis is limited. We compared the efficacy and tolerability of the aromatase inhibitor letrozole combined with norethisterone acetate versus norethisterone acetate alone in treating pain symptoms.

**METHODS:** This prospective, open-label, non-randomized trial included 82 women with pain symptoms caused by rectovaginal endometriosis. Patients received either a combination of letrozole and norethisterone acetate (group L) or norethisterone acetate alone (group N) for 6 months. Changes in pain symptoms during treatment and in the 12 months of follow-up were evaluated. Side effects of each treatment protocol were recorded.

**RESULTS:** Intensity of chronic pelvic pain and deep dyspareunia significantly decreased during treatment ( $P < 0.001$  versus baseline by 3 months) in both study groups. At both 3- and 6-month assessment, the intensity of chronic pelvic pain ( $P < 0.001$ ,  $P = 0.002$ , respectively) and deep dyspareunia ( $P < 0.001$ ,  $P = 0.005$ , respectively) was significantly lower in group L than group N. At completion of treatment, 63.4% of women in group N were satisfied with treatment compared with 56.1% in group L ( $P = 0.49$ ). Pain symptoms recurred after the completion of treatment; at 6-month follow-up no difference was observed in the intensity of pain symptoms between the groups. Adverse effects were more frequent in group L than in group N ( $P = 0.02$ ).

**CONCLUSIONS:** The combination drug regimen was more effective in reducing pain and deep dyspareunia than norethisterone acetate; however, letrozole caused a higher incidence of adverse effects, cost more and did not improve patients' satisfaction or influence recurrence of pain.

**Key words:** aromatase inhibitors / endometriosis / letrozole / norethisterone acetate / prospective study

## Introduction

Rectovaginal endometriosis usually causes pain symptoms such as dysmenorrhea, deep dyspareunia and chronic pelvic pain. Although the complete excision of rectovaginal endometriotic nodules may determine a significant improvement in pain symptoms (Donnez *et al.*, 1995; Anaf *et al.*, 2001; Ford *et al.*, 2004; Kristensen and Kjer 2007), this surgery may be technically demanding and deep

endometriotic lesions may not be completely excised by inexperienced surgeons. In addition, these surgical procedures are associated with the risk of severe urological and colorectal complications (Slack *et al.*, 2007), which may not be easily accepted by young women undergoing surgery because of a benign disease.

When pregnancy is not desired, hormonal therapies may be administered to symptomatic patients who previously underwent non-radical interventions and want to avoid further surgery. Current

medical therapies do not eradicate endometriotic lesions but they have been demonstrated to efficaciously and safely relieve pain symptoms (Vercellini et al., in press). In the last 20 years, our understanding of the pathogenesis of endometriosis at the molecular and cellular level has significantly improved. Based on these molecular findings, new medical therapies for endometriosis have been developed (Ferrero et al., 2005).

Aromatase P450 is a key enzyme for estrogen biosynthesis, catalysing the conversion of androstenedione and testosterone to estrone and estradiol ( $E_2$ ) via hydroxylation. Since the late 1990s, several studies using either PCR or immunohistochemistry demonstrated the expression of aromatase P450 in both eutopic and ectopic endometrium from patients with endometriosis, but not in eutopic endometrium from disease-free women and in endometriosis-free peritoneal tissue (Noble et al., 1996; Kitawaki et al., 1997; Kitawaki et al., 1999; Wöfler et al., 2005; Velasco et al., 2006; Hudelist et al., 2007; Bukulmez et al., 2008; Smuc et al., 2009). It was also demonstrated that estrogen stimulates the production of cyclooxygenase type 2 enzyme, resulting in elevated levels of prostaglandin  $E_2$ , which is a potent stimulator of aromatase activity (Zeitoun et al., 1999). Based on these observations, it was hypothesized that there might be a positive feedback loop in favour of continuous local production of estradiol in endometriotic tissue (Zeitoun et al., 1999). However, this hypothesis and previous studies were recently contradicted by two publications. Colette et al. (in press) demonstrated that aromatase protein is undetectable by immunohistochemistry in endometriotic lesions and in the eutopic endometrium of women with endometriosis. In addition, by using quantitative PCR, it was shown that the expression of the aromatase gene is low in endometriomas and barely detectable in only a small percentage of eutopic endometrial samples, peritoneal lesions and rectovaginal nodules (Colette et al., in press). In line with these observations, another study reported the absence of aromatase activity in eutopic and ectopic tissue obtained from patients with endometriosis (Delvoux et al., 2009). In addition, this study showed that endometriotic lesions are capable of creating a hyperestrogenic environment, which is not caused by changes in the aromatase activity but is the result of increased reduction of estrone into  $E_2$  and decreased oxidation of  $E_2$  into estrone (Delvoux et al., 2009).

Aromatase inhibitors are widely used in the treatment of patients with hormone-dependent breast cancer. Over the last 10 years, aromatase inhibitors have been proposed for the treatment of endometriosis both in post-menopausal women (Takayama et al., 1998; Razzi et al., 2004; Fatemi et al., 2005; Mousa et al., 2007) and in subjects of reproductive age (Ailawadi et al., 2004; Shippen and West, 2004; Amsterdam et al., 2005; Verma and Konje 2009). Aromatase inhibitors interfere with estrogen production in the ovaries and at the periphery. When these agents are administered as monotherapy to premenopausal women, the reduction of estrogen production causes an increase in gonadotrophin secretion because of the reduced feedback of estrogen to the hypothalamus and pituitary. Gonadotrophins cause a stimulation of ovarian function (de Ziegler et al., 2005). Administration of the aromatase inhibitor letrozole to cycling female rats caused an increase in ovarian weight (Sinha et al., 1998). In a pilot study including 12 women with endometriosis, the daily oral administration of letrozole 2.5 mg and desogestrel 75  $\mu$ g resulted in the formation of functional ovarian cysts in all the study subjects

(Remorgida et al., 2007b). The development of functional ovarian cysts was also observed in over 50% of the patients with symptomatic uterine myomas treated with letrozole monotherapy for 3 months (Gurates et al., 2008). Therefore, when aromatase inhibitors are continuously administered for several months to women of reproductive age, they should be combined with additional drugs that effectively down-regulate the ovaries and gonadal  $E_2$  biosynthesis. These double-drug regimens suppress both ovarian and extraovarian  $E_2$  production aiming to obtain the deepest possible hypo-estrogenism. Case reports and pilot studies combined type II aromatase inhibitors (anastrozole and letrozole) with ovarian suppressive agents (such as progestins and oral contraceptive pill) (Ailawadi et al., 2004; Amsterdam et al., 2005; Remorgida et al., 2007a; Remorgida et al., 2007b). Some of these studies suggested that aromatase inhibitors not only reduce pain symptoms but that they also eradicate the disease either as an alternative to surgery (Ailawadi et al., 2004; Shippen and West, 2004) or as a post-operative prevention of recurrence (Soysal et al., 2004). However, the available data on the effectiveness of aromatase inhibitors in treating pain symptoms related to the presence of endometriosis is still limited. In fact, published studies had small sample sizes and reported only short-term follow-up after the administration of aromatase inhibitors (Ferrero et al., 2009).

Given this background, this prospective, non-randomized, open-label study sought to determine whether the efficacy and tolerability of a combination of an aromatase inhibitor and a progestogen (experimental treatment) was superior to a low-dose progestogen (chosen as reference treatment) in treating pain symptoms related to the presence of rectovaginal endometriosis.

## Materials and Methods

This prospective, non-randomized, open-label trial compared the efficacy of norethisterone acetate alone or in combination with the aromatase inhibitor letrozole in the treatment of pain symptoms related to the presence of rectovaginal endometriosis.

Subjects of the study had undergone laparoscopy or laparotomy for symptomatic endometriosis in other hospitals but deep endometriotic lesions were not excised. These patients had persistent pain symptoms after surgery and were referred to our endometriosis centre. Patients included in the study had pain symptoms of more than 12-month duration and did not want to undergo further surgery. Only premenopausal women were included in the study.

The diagnosis of endometriosis was based on vaginal and rectal examinations performed by one of the authors (V.R.), transvaginal ultrasonography, rectal water contrast transvaginal ultrasonography (Valenzano Menada et al., 2008a, b), and histological examination of the specimens excised during previous surgery. Patients with gastrointestinal complaints suggestive of bowel endometriosis underwent multidetector computerized tomography enteroclysis (Biscaldi et al., 2007; Biscaldi et al., 2009). Kidney and urinary tract evaluation were always performed.

The exclusion criteria for the study were: uropathy or endometriotic nodules infiltrating the muscular layer of the bowel wall; ovarian endometrioma of diameter  $>3$  cm; therapies for endometriosis other than non-steroidal anti-inflammatory drugs (NSAID) in the 3 months before inclusion in the study (6 months for GnRH analogues); previous use of aromatase inhibitors; unwillingness to tolerate menstrual changes; undiagnosed vaginal bleeding; osteopenia or osteoporosis; current or past history of seizure disorders; pulmonary, cardiac, hepatic or renal diseases;

thromboembolic or cerebrovascular events; pregnancy; psychiatric disturbances and history of drug or alcohol abuse.

Eligible patients were offered one of the following therapies for 6 months: oral norethisterone acetate (2.5 mg/day, Primolut-Nor; Schering, Milan, Italy; group N) or a combination of oral letrozole (2.5 mg/day, Femara; Novartis Farma, Varese, Italy), norethisterone acetate (2.5 mg/day), elemental calcium (1000 mg/day) and vitamin D3 (880 IU/day, Cacit-Vitamina D3; Procter & Gamble, Rome, Italy; group L). The treatment was started on the first day of menstruation.

The patients included in the study were informed that medical treatments are effective in relieving pain symptoms caused by endometriosis, but that pain symptoms often recur to a degree similar to that at baseline after treatments are discontinued (Vercellini *et al.*, in press). Therefore, the administration of a 6-month medical therapy should not be considered definitively curative of endometriosis. Patients were informed that both treatments have previously been demonstrated to significantly reduce the intensity of pain symptoms caused by endometriosis (Ailawadi *et al.*, 2004; Vercellini *et al.*, 2005; Remorgida *et al.*, 2007a); in addition, they were told that it is unknown whether adding letrozole to norethisterone acetate may determine a further decrease in the severity of pain symptoms. Patients were informed that some preliminary studies suggested that aromatase inhibitors might cause the disappearance of endometriotic lesions (Ailawadi *et al.*, 2004; Shippen and West, 2004), but these observations were not confirmed at our hospital (Remorgida *et al.*, 2007a). Patients were also informed that norethisterone acetate is approved by the Italian Ministry of Health for the treatment of endometriosis; on the contrary, the administration of letrozole should be considered experimental. Finally, patients were counselled regarding the potential side effects of the treatment. They were told that norethisterone acetate might cause irregular uterine bleeding, weight increase and decreased libido (Vercellini *et al.*, 2005). They were also informed that, in previous pilot studies, we observed that aromatase inhibitors might cause bone and joint pain, myalgia and headache (Remorgida *et al.*, 2007a, b). The costs of the two treatment protocols were provided.

Treatment allocation was decided on the basis of the preference of the patients; patients were recruited in each study arm until the desired sample size was achieved.

The subjects of the study were allowed to take NSAIDs when needed (naproxen sodium, 550 mg tablet, Synflex Forte 550, Recordati Industria Chimica e Farmaceutica, Milan, Italy); however, they were asked to record the number of tablets used each month during treatment.

The primary end-point of the study was to compare the changes in pain symptoms after 6-month treatment with the two study protocols. The secondary objective of the study was to evaluate the course of pain symptoms in the 12 months after the interruption of treatment. Side effects of each treatment protocol were also determined.

Each patient was asked to complete a questionnaire on the presence and severity of dysmenorrhea, non-menstrual pelvic pain and deep dyspareunia. The severity of pain symptoms was measured using a 10-cm visual analogue scale (VAS), the left extreme of the scale indicating the absence of pain, and the right indicating the pain as bad as it could be. A score of 0.1–5.0 was considered mild pain, 5.1–8.0 moderate pain and 8.1–10.0 severe pain. Patients enrolled in the study had at least one moderate or severe symptom.

Study subjects had monthly consultations during the 6-month treatment and consultations at 3, 6 and 12 months after the completion of treatment. The intensity of pain symptoms was evaluated before starting the treatment, after 3 and 6 months of treatment and at 3, 6 and 12 months after interruption of the treatment.

After the completion of treatment, the women rated the overall degree of satisfaction with their treatment by answering the following question: 'Taking into consideration the variations in pain symptoms, in overall

well-being and quality of life, as well as the adverse effects experienced, if any, how would you define the level of satisfaction with your treatment?' as previously described by other authors (Vercellini *et al.*, 2005). Answers were based on a 5-point Likert scale (very satisfied, satisfied, uncertain, dissatisfied, very dissatisfied).

Complete blood count, serum electrolytes, kidney and liver function tests and a lipid profile were performed before the onset of therapy, monthly during treatment and at the completion of treatment. A bone densitometry determination of the hip and lumbar spine (by dual-energy X-ray absorptiometry or DEXA scan) was performed within 1 month before the onset of the study and was repeated within 1 month after completion of the treatment in group L. Adverse effects experienced during the 6-month treatment were recorded during monthly consultations. Uterine bleeding was defined as spotting (scanty bleeding not requiring usual sanitary protection); breakthrough bleeding (light or moderate bleeding requiring sanitary protection) and metrorrhagia (more than normal menstruation). The cost of 6-month treatment with the two regimens used in the study was determined.

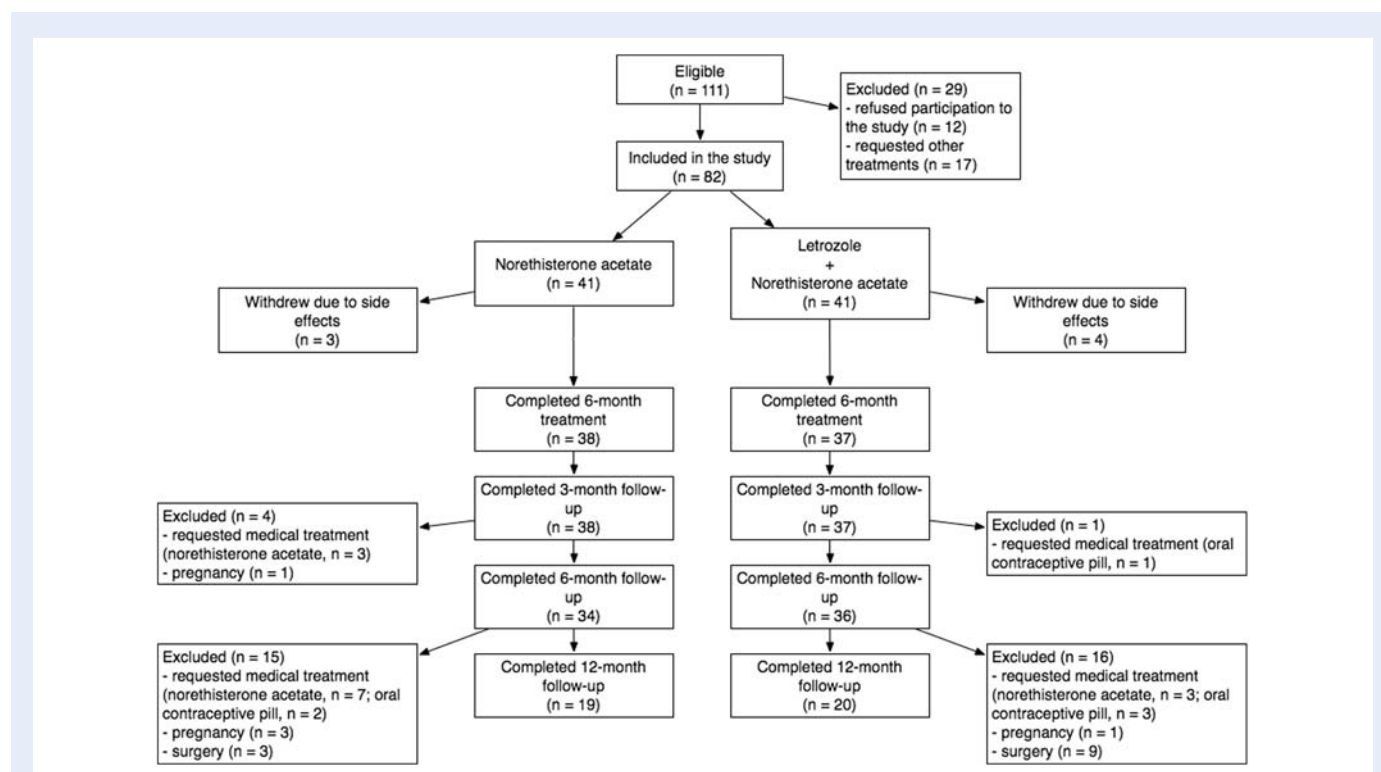
The local Institutional Review Board approved the study protocol. The patients enrolled in the study signed a written informed consent.

## Statistical analysis

In calculating the sample size required, it was hypothesized that about 65% of patients should be satisfied after a 6-month treatment with norethisterone acetate. As the double-drug regimen investigated in the study included the administration of norethisterone acetate at the same dosage, we expected the percentage of satisfied patients to be higher in group L (1-sided test). A difference of 25% in satisfaction rate between the study groups was considered clinically relevant. To have an 80% chance of detecting such a difference at an overall statistical significance level of 5%, 34 patients per group were required. Allowing for dropouts, the aim was to recruit a total of about 80 women. Categorical variables were compared by using the chi-square test. The comparison of pain intensity between the two study groups was performed by using the Student's *t*-test and the Mann–Whitney *U*-test according to the data distribution. Variations in grading of symptoms between baseline and follow-up were compared by using the Student's *t*-test and the Signed Rank test according to the data distribution.  $P < 0.05$  was considered statistically significant. Data were analysed using the Sigma Stat software version 3.5 and the SPSS software version 13.0 (SPSS Science, Chicago, IL, USA).

## Results

One hundred and eleven women referred to our endometriosis outpatient clinic were eligible for the study; 12 women refused participation to the study and 17 women requested other treatments (oral contraceptive pill,  $n = 13$ ; GnRH analogues,  $n = 2$ ; desogestrel-only contraceptive pill,  $n = 2$ ). Therefore, 82 patients were included in the study. Three women in group N and four women in group L withdrew because of adverse effects. All these patients were classified as having treatment failure in the evaluation of efficacy of treatment (the primary end-point), but were excluded from the evaluation of symptom course after 6-month treatment (secondary end-point). None of the patients was lost during the study period. The diagrammatic flow of the participants is given in Fig. 1. Table I shows the demographic characteristics of the patients included in the study; the distribution of the considered variables was similar in the two study groups.



**Figure 1** Flow chart showing recruitment and women's progress through the study of treatments for pain associated with rectovaginal endometriosis.

**Table 1** Demographic characteristics of the patients included in the study of treatments for pain associated with rectovaginal endometriosis

Variable	Norethisterone acetate group		Norethisterone acetate and letrozole group		P
	n	%	n	%	
Age					
<30 years	15	36.6	17	41.5	0.651
≥30 years	26	63.4	24	58.5	
Parity					
0	29	70.7	30	73.2	0.806
≥1	12	29.3	11	26.8	
BMI (kg/m <sup>2</sup> )					
<18.49	3	7.3	1	2.4	0.208
18.50–24.99	34	82.9	39	95.1	
25.00–29.99	4	9.8	1	2.4	

<sup>a</sup>Body mass index = weight (kg)/height (m)<sup>2</sup>.

At the 6-month assessment, 8 (19.5%) women in group N were very satisfied with their treatment, 18 (43.9%) were satisfied, 5 (12.2%) were uncertain, 6 (14.6%) were dissatisfied and 4 (9.8%) were very dissatisfied, while corresponding figures for group L were, respectively, 7 (17.1%), 16 (39.0%), 6 (14.6%), 7 (17.1%) and 5 (12.2%). This analysis included all patients enrolled in the study.

Overall, 63.4% (26/41) of the women in group N were satisfied or very satisfied after 6 months of treatment compared with 56.1% (23/41) in group L ( $P = 0.499$ ).

When compared with baseline values, the intensity of chronic pelvic pain was significantly decreased at 3-month treatment both in group N ( $P < 0.001$ ) and in group L ( $P < 0.001$ ); the intensity of chronic pelvic

pain continued to decrease during treatment and at 6-month treatment it was significantly lower than at 3-month treatment both in group N ( $P = 0.001$ ) and in group L ( $P = 0.008$ ). Similarly, the intensity of deep dyspareunia was significantly decreased at 3-month treatment both in group N ( $P < 0.001$ ) and in group L ( $P < 0.001$ ); at 6-month treatment, the intensity of deep dyspareunia was significantly lower than at 3-month treatment both in group N ( $P = 0.002$ ) and in group L ( $P = 0.008$ ).

Table II shows that the intensity of chronic pelvic pain and deep dyspareunia was significantly lower at both 3- and 6-month treatment in group L than in group N. At the completion of treatment, two women in group N (7.7%) and one woman in group L (3.3%) had moderate chronic pelvic pain ( $P = 0.470$ ). Four women in group N had either moderate ( $n = 3$ ) or severe deep dyspareunia ( $n = 1$ ), while only one patient in group L had moderate deep dyspareunia ( $P = 0.161$ ).

In all patients, menstrual cycles resumed within 2 months after cessation of the medication. At 3-month follow-up after the completion of treatment, the intensity of dysmenorrhea, deep dyspareunia and chronic pelvic pain was significantly decreased when compared with baseline values in both study groups (Fig. 2). Pain symptoms quickly recurred and at 6- and 12-month follow-up no significant difference was observed in the intensity of pain symptoms between the two study groups (Table II).

The mean ( $\pm$ SD) number of naproxen sodium tablets used per patient each month throughout the study period was 4.2 ( $\pm$ 2.1) in

21 patients in group N and 3.1 ( $\pm$ 1.6) in 16 patients in group L ( $P = 0.116$ ).

Three patients included in group N and four patients included in group L withdrew from the study because of adverse effects ( $P = 0.693$ ). In group N, two patients had breakthrough bleeding and one patient had severe migraine attacks. In group L, two patients interrupted the treatment because of severe joint pain, one patient had severe migraine attacks and one patient suffered myalgia and breakthrough bleeding.

Among the patients who completed the treatment protocol, 7 women in group N (18.4%) and 16 women in group L reported at least one adverse effect (43.2%;  $P = 0.020$ ). The adverse effects reported by the subjects of the study were breakthrough bleeding (group N,  $n = 1$ ; group L,  $n = 1$ ), spotting (group N,  $n = 3$ ; group L,  $n = 4$ ), migraine (group N,  $n = 2$ ; group L,  $n = 1$ ), joint pain (group L,  $n = 5$ ), myalgia (group L,  $n = 5$ ), depression (group N,  $n = 1$ ; group L,  $n = 1$ ), hair loss (group L,  $n = 1$ ) and decreased libido (group L,  $n = 1$ ). Weight increase during treatment was reported by 7 women in group N (18.4%) and by 8 women in group L (21.6%;  $P = 0.729$ ). The mean ( $\pm$ SD) gain in patients reporting a weight increase was 2.8  $\pm$  0.8 kg in group N and 2.9  $\pm$  0.9 kg in group L ( $P = 0.737$ ). There were no adverse effects on blood count, liver function, renal function and lipid profile. DEXA scans showed that during treatment with letrozole, there was no significant change in the mineral bone density both in the lumbar spine and in the hip (data not

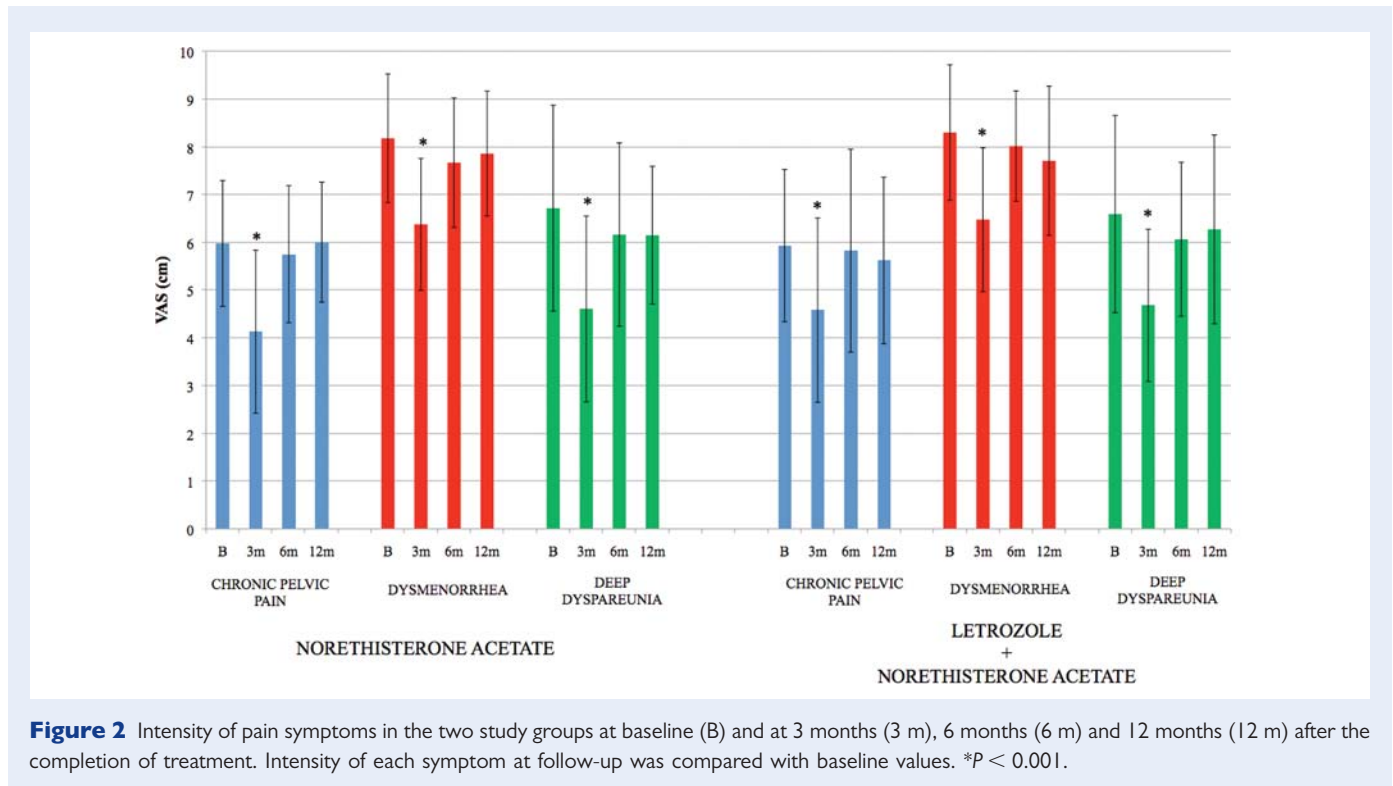
**Table II** Intensity of pain symptoms\* at baseline, at 3 and 6 months during treatment and at 3, 6 and 12 months after completion of treatment (follow-up)

	Norethisterone acetate (group N)	Norethisterone acetate and letrozole (group L)	P (Group N versus L)
<b>Dysmenorrhea</b>			
Baseline	8.2 $\pm$ 1.4 ( $n = 36$ )	8.3 $\pm$ 1.4 ( $n = 35$ )	0.619
3 months follow-up	6.4 $\pm$ 1.4 ( $n = 36$ )	6.5 $\pm$ 1.5 ( $n = 35$ )	0.773
6 months follow-up	7.7 $\pm$ 1.4 ( $n = 32$ )	8.0 $\pm$ 1.2 ( $n = 34$ )	0.265
12 months follow-up	7.9 $\pm$ 1.3 ( $n = 19$ )	7.7 $\pm$ 1.6 ( $n = 19$ )	0.742
<b>Chronic pelvic pain</b>			
Baseline	6.0 $\pm$ 1.3 ( $n = 26$ )	5.9 $\pm$ 1.6 ( $n = 30$ )	0.905
3 months of treatment	3.4 $\pm$ 1.8 ( $n = 26$ )	1.9 $\pm$ 1.3 ( $n = 30$ )	<0.001
6 months of treatment	2.8 $\pm$ 1.7 ( $n = 26$ )	1.5 $\pm$ 1.4 ( $n = 30$ )	0.002
3 months follow-up	4.1 $\pm$ 1.7 ( $n = 26$ )	4.6 $\pm$ 1.9 ( $n = 30$ )	0.360
6 months follow-up	5.8 $\pm$ 1.4 ( $n = 22$ )	5.8 $\pm$ 2.1 ( $n = 29$ )	0.987
12 months follow-up	6.0 $\pm$ 1.3 ( $n = 14$ )	5.6 $\pm$ 1.7 ( $n = 17$ )	0.751
<b>Deep dyspareunia<sup>a</sup></b>			
Baseline	6.7 $\pm$ 2.1 ( $n = 30$ )	6.6 $\pm$ 2.1 ( $n = 30$ )	0.750
3 months of treatment	3.8 $\pm$ 1.9 ( $n = 30$ )	2.2 $\pm$ 1.4 ( $n = 30$ )	<0.001
6 months of treatment	3.1 $\pm$ 2.2 ( $n = 30$ )	1.7 $\pm$ 1.1 ( $n = 30$ )	0.005
3 months follow-up	4.6 $\pm$ 1.9 ( $n = 30$ )	4.7 $\pm$ 1.6 ( $n = 30$ )	0.868
6 months follow-up	6.2 $\pm$ 1.9 ( $n = 26$ )	6.1 $\pm$ 1.6 ( $n = 30$ )	0.835
12 months follow-up	6.2 $\pm$ 1.4 ( $n = 17$ )	6.3 $\pm$ 2.0 ( $n = 17$ )	0.873

<sup>a</sup>34 women included in group N and 34 women included in group L were sexually active at the time of the study.

\*Severity of pain symptoms was measured using a 10-cm VAS. A score of 0.1–5.0 was considered mild pain, 5.1–8.0 moderate pain and 8.1–10.0 severe pain.





**Figure 2** Intensity of pain symptoms in the two study groups at baseline (B) and at 3 months (3 m), 6 months (6 m) and 12 months (12 m) after the completion of treatment. Intensity of each symptom at follow-up was compared with baseline values. \* $P < 0.001$ .

shown). No woman fell into the category of osteopenia at the conclusion of the treatment.

In Italy, the cost of 6-month treatment with norethisterone acetate alone was 8.5 euros per patient, while the treatment with letrozole and norethisterone acetate cost 1051.7 euros. When calcium and vitamin D were added to the double-drug regimen, the cost increased to 1083.7 euros per patient.

## Discussion

Previous case reports and pilot studies investigated the effectiveness of aromatase inhibitors in treating pain symptoms related to the presence of endometriosis (Nawathe *et al.*, 2008; Ferrero *et al.*, 2009). In particular, the combination of letrozole and norethisterone acetate was shown to be effective in treating pain symptoms caused by endometriosis (Ailawadi *et al.*, 2004; Remorgida *et al.*, 2007a); however, the use of a double-drug regimen did not allow to determine the effect of each drug on the improvement of pain symptoms. Based on these considerations, we designed this prospective study comparing letrozole combined with norethisterone acetate, and norethisterone acetate alone, in the treatment of pain symptoms caused by endometriosis.

The findings of the current study confirm previous reports by other authors (Ailawadi *et al.*, 2004; Shippen and West, 2004; Amsterdam *et al.*, 2005; Hefler *et al.*, 2005) and by us (Remorgida *et al.*, 2007a, b) demonstrating that aromatase inhibitors quickly improve pain symptoms related to the presence of endometriosis. In fact, after 3 months of treatment, the intensity of chronic pelvic pain and deep dyspareunia was significantly decreased when compared with baseline values. For the first time, this study demonstrated that, at 3 and 6 months of

treatment, the intensity of chronic pelvic pain and deep dyspareunia were significantly lower in patients receiving letrozole and norethisterone acetate than in those receiving norethisterone acetate alone.

A low dose of norethisterone acetate (2.5 mg/day) was chosen as the reference treatment. Norethisterone acetate has previously been shown to be effective in treating pain symptoms in women with endometriosis. In the 1990s, a retrospective study showed that the administration of norethisterone acetate at a dosage between 5 and 20 mg/day relieves dysmenorrhea and non-cyclical pelvic pain (Muneyyirci-Delale and Karacan, 1998). More recently, a prospective randomized trial including 90 women with rectovaginal endometriosis showed that the administration of low-dose norethisterone acetate (2.5 mg/day) significantly improves dysmenorrhea, deep dyspareunia, chronic pelvic pain and dyschezia (Vercellini *et al.*, 2005).

Aromatase inhibitors have been widely used in post-menopausal patients with breast cancer; however, the experience of their use in women of reproductive age is still limited. A potential concern related to the administration of aromatase inhibitors to women of reproductive age is the adverse effects that these drugs might cause. In the present study, a significantly higher percentage of adverse effects was experienced by women under the double-drug regimen than in those receiving only the low-dose progestin. In particular, 17.1% of the patients receiving letrozole experienced joint pain; in two cases the intensity of this adverse effect was severe and the patients interrupted the treatment. 14.3% of the patients receiving letrozole reported myalgia and one patient using the aromatase inhibitor had hair loss. These adverse effects of aromatase inhibitors have previously been reported both in breast cancer patients (Carlini *et al.*, 2003; Felson and Cummings 2005; Burstein 2007; Sestak *et al.*, 2008) and in women with endometriosis (Remorgida *et al.*,

2007a, b; Verma and Konje 2009). Bone mineral density was not reduced by the treatment protocol, confirming previous observations in premenopausal women with endometriosis receiving aromatase inhibitors (Ailawadi *et al.*, 2004; Amsterdam *et al.*, 2005; Remorgida *et al.*, 2007a). Patients included in group L received calcium and vitamin D; however, it is unknown whether this supplementation is required. In fact, norethisterone acetate may have a positive effect on bone metabolism (Riis *et al.*, 2002). In this study, calcium and vitamin D were administered to women receiving letrozole because previous investigations demonstrated that this multiple drugs regimen does not have negative effects on mineral bone density (Ailawadi *et al.*, 2004; Remorgida *et al.*, 2007a). Although the double-drug regimens including aromatase inhibitors and add-back progestins or oral contraceptives do not appear to reduce mineral bone density after 6 months of treatment, no data are available on the long-term use of these regimens in premenopausal women. Patients included in group N did not undergo DEXA scans and did not receive supplementation with calcium and vitamin D because there is no evidence that norethisterone acetate has a detrimental effect on bone metabolism (Riis *et al.*, 2002).

Currently available hormonal therapies do not eradicate endometriotic lesions, and pain relapse at drug withdrawal is commonly observed (Vercellini *et al.*, in press). However, previous case reports and pilot studies hypothesized that aromatase inhibitors may eradicate endometriotic lesions suggesting that they might represent a definitive 'cure' for endometriosis (Ailawadi *et al.*, 2004; Shippen and West, 2004). These preliminary findings were not confirmed in the current study. Recurrence of pain symptoms to a degree similar to that at baseline was observed in both study groups after interruption of treatment. In addition, the presence of endometriotic lesions was confirmed in women who underwent surgery after the completion of treatment with letrozole. These observations demonstrate that aromatase inhibitors do not eradicate endometriotic lesions, confirming previous findings by our group (Remorgida *et al.*, 2007a).

Obviously, the adverse effects of treatment may affect the degree of satisfaction of the patients and may cause interruption of therapy. In the current study, although the intensity of pain symptoms during treatment was lower in group L than in group N, no significant difference was observed in the degree of satisfaction of the women with treatment. It is possible that the higher incidence of adverse effects in group L decreased the satisfaction of the patients. Since pain relapse is expected after the interruption of hormonal treatments, not only efficacy but also patients' satisfaction, incidence of adverse effects, and costs should be considered when choosing a therapy which might be used for years by patients with endometriosis (Vercellini *et al.*, in press). The findings of this study are in line with the previous observation by other authors that progestogens are safe, well tolerated and effective in the long-term treatment of pain symptoms caused by deep endometriosis (Vercellini *et al.*, in press). In particular, norethisterone acetate represents an adequate alternative to surgery in symptomatic patients who do not desire pregnancy and do not have obstructive uropathy, bowel stenosis and adnexal masses of doubtful nature. The recent emphasis on the use of aromatase inhibitors in endometriosis should be tempered by failure of definitively curing the disease, suboptimal tolerability and cost.

Some limitations characterize the present study. Firstly, women were not randomized to receive one of the two study protocols

because patients receiving letrozole covered the cost of this drug. Therefore, economic issues may have influenced the decision of the patients to receive one of the treatment protocols. However, it seems unlikely that this study design may have biased the findings of the study, in fact it should be noted that the baseline intensity of pain symptoms was similar between the two study groups. Another potential limitation of the study is the length of the treatment. Endometriosis is a chronic disease (Giudice and Kao, 2004) and thus it may require a chronic therapy. However, the scheduled treatment period was 6 months, for two reasons. Based on the fact that previous studies suggested that aromatase inhibitors might cause the regression of endometriotic lesions (Ailawadi *et al.*, 2004; Shippen and West, 2004), we wanted to determine whether the administration of aromatase inhibitors could guarantee a longer pain-free interval after the interruption of treatment. Secondly, the safety of a long-term administration of aromatase inhibitors to women of reproductive age remains to be established.

In conclusion, the current prospective, open-label, non-randomized trial demonstrates that both letrozole combined with norethisterone acetate and norethisterone acetate alone are effective in treating pain symptoms related to the presence of rectovaginal endometriosis. The administration of aromatase inhibitors with norethisterone acetate may result in a greater reduction in the intensity of pain symptoms during treatment; however, the satisfaction of the patients was similar in the two study groups. After the interruption of treatment, pain symptoms recurred both in patients treated with the double-drug regimen and in those receiving norethisterone acetate alone. The administration of aromatase inhibitors was associated with a higher incidence of adverse effects; furthermore, some of these side effects (such as joint pain and myalgia) were not experienced during treatment with norethisterone acetate alone. On the basis of these findings and considering the higher cost of the double-drug regimen, we believe that aromatase inhibitors should be administered only to patients who previously failed to respond to established therapies (such as progestins or oral contraceptive pill) and refuse surgery. Patients receiving aromatase inhibitors should be informed that adverse effects of variable severity occur in about 40% of the treated patients.

## Author's Role

R.V. had the original idea of the study. S.R., R.N. and V.P.L supervised the whole study procedure including the design of the study and interpretation of results. F.S., C.G., N.R. and R.V. recruited the patients enrolled in the study and performed the follow-up. F.S. performed the statistical analyses and prepared the draft of the manuscript. S.R., R.V. and V.P.L. revised the manuscript.

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- Submitted on June 28, 2009; resubmitted on July 25, 2009; accepted on August 4, 2009