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Accuracy of anogenital distance and anti-Müllerian hormone in the diagnosis of endometriosis without surgery

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Synopsis: Anti-Müllerian hormone and anogenital distance were found to be useful in the diagnosis of endometriosis.

ABSTRACT

Objective: To assess the predictive ability of a combination of anogenital distance (AGD) and anti-Müllerian hormone (AMH) to diagnosis the presence of endometriosis without surgery.

Methods: The present study included women diagnosed with endometriosis and a control group who attended the “Virgen de la Arrixaca” University Hospital, Murcia, Spain, between September 1, 2014, and May 31, 2015. Serum concentrations of AMH were measured, and two AGD measurements were obtained: from the anterior clitoral surface to the upper verge of the anus (AGD_{AC}), and from the posterior fourchette to the upper verge of the anus (AGD_{AF}). Data were assessed by receiver operator characteristic (ROC) curves.

Results: Women in the endometriosis group (n=57) had significantly shorter AGD_{AF} (22.8±4.6 vs 27.2±5.7 mm; *P*<0.001) and lower AMH (2.2±2.5 vs 3.3±1.9 ng/mL; *P*<0.003) compared with the control group (n=93). Women with serum AMH below the clinical cut-off (1 ng/mL) were 17.40-times more likely to have endometriosis (95% confidence interval [CI] 5.64–53.82). The area under the ROC curve of combined AMH and AGD_{AF} was 0.77 (95% CI 0.70–0.85).

Conclusion: The model for predicting endometriosis on the basis of AMH and AGD could be useful for clinicians and epidemiologists to improve diagnosis and prognosis of this condition.

1 INTRODUCTION

Endometriosis is an estrogen-dependent disease defined as the presence of endometrial tissue functionally active outside the uterine cavity [1]. It can damage many organs, but the most frequently affected one is the ovary. There are different types of endometriosis, including endometriotic cysts (endometriomas), deep infiltrating endometriosis (DIE), and superficial implants [1]. Endometriosis has a prevalence of approximately 10%, affecting 4%–30% of women of reproductive age [2], and is usually associated with infertility [3]. Between 30% and 50% of women with endometriosis have reproductive problems; this percentage rises to 70%–80% if pelvic pain is present [4].

One of the challenges in endometriosis is its early diagnosis among patients with clinical disease. Standard diagnosis is carried out by direct visualization and examination of histologic lesions; however, histologic samples are not always

available and this approach may not be feasible for early stages of the disease or for screening a healthy population [5]. Although several predictive models for diagnosis of endometriosis have been proposed [6], none of them has been widely applied. In general, the models have been designed for a specific subtype of endometriosis [7].

Circulating anti-Müllerian hormone (AMH) seems to be the best biochemical marker of ovarian function, owing to its ability to reflect the number of antral and pre-antral follicles present in the ovaries [8]. It has been applied to various clinical situations, such as monitoring the response to ovarian stimulation in assisted reproductive techniques, timing of menopause, and iatrogenic damage to the ovarian follicle reserve [8]. It has also been used to assess ovarian reserve depletion after surgery for endometriomas [9], and as a follow-up biomarker for women with histologically confirmed severe endometriosis (stages III–IV) [10,11]. However, the influence of endometriosis itself on ovarian reserve remains controversial [9,12].

Anogenital distance (AGD) is thought to be an accurate marker of the prenatal hormonal milieu and potential intra-uterus insults. A relationship between shorter AGD and the presence of endometriosis, both endometriomas and DIE, has recently been reported [13] supporting the idea that this disease has a potential intrauterine origin. Moreover, AGD has been shown to be a suitable diagnostic tool in endometriosis [14].

Individually, both AGD and AMH have been proposed as predictors of the presence of endometriosis. Both determinations are non-invasive and, if shown to be useful in clinical practice, they might help to improve management of endometriosis. The aim

of the present study was therefore to assess the ability of the combined measures to predict the presence of endometriosis.

2. MATERIAL AND METHODS

The present study compared data between women with endometriosis and a control group at the Department of Obstetrics and Gynecology, Clinical University Hospital "Virgen de la Arrixaca," Murcia, Spain, between September 1, 2014, and May 31, 2015. The study was approved by the Ethics Research Committee of the University of Murcia, Espinardo, Spain (no. 770/2013; October 3, 2013). Written informed consent was obtained from all participants.

The inclusion criterion for the endometriosis group was attendance at the endometriosis unit of the study hospital, including prevalent and incident endometriosis. The exclusion criteria were pregnancy, previous surgery for endometriosis, oncologic treatment, and genitourinary prolapse. Endometriosis was diagnosed by clinical interview and confirmed by symptoms, signs, and transvaginal ultrasonography (TVUS) findings [15]. Endometriosis was further classified as endometriomas or DIE. The control group included women without endometriosis were recruited during routine gynecologic visits. For both groups, consecutive women attending the clinic were recruited.

During the study visit, all participants were interviewed by two gynecologists using an established method. They completed health questionnaires, including age and gynecologic and obstetric history, and underwent a gynecologic examination with TVUS (Voluson E-8 and 4–9-MHz transducer, General Electric Healthcare, Chicago,

IL, USA). Height and weight were recorded by using an SC 330-S digital scale (Tanita, London, UK), and body mass index (BMI, calculated as weight in kilograms divided by the square of height in meters) was determined. Endometriosis-associated pelvic pain (including dysmenorrhea, chronic pelvic pain, dyspareunia, dysuria, and dyschezia) was evaluated by using a visual analogue scale from 0 to 10 [16]. Uterine and ovarian morphology was assessed by TVUS.

Anogenital distance was measured by using a stainless steel digital caliper (VWR International, West Chester, PA, USA). Each woman lay in the lithotomy position with her thighs at a 45° angle to the examination table. Two distances were measured: AGD_{AC}, from the clitoral surface to the upper verge of the anus; and AGD_{AF}, from the posterior fourchette to the upper verge of the anus (Figure S1). To improve precision, two gynecologists who were masked to the clinical evaluation of the women measured each distance three times and mean values were used in the analyses. Both the gynecologists and the support staff were also masked to the participant's gynecologic condition. Although both distances were measured, a previous study indicated that AGD_{AF} was better at discriminating the presence of endometriosis [14], therefore, only AGD_{AF} was included in the data analysis.

A non-fasting blood sample was drawn between 08:00 and 10:00 on the same day as the clinical appointment, which was selected to coincide with the early follicular phase of the menstrual cycle (days 2–5). Serum concentrations of AMH were measured at the study hospital's laboratory by enzyme-linked immunosorbent assay (Elecsys AMH assays; Roche Diagnostics, Mannheim, Germany). The intra- and inter-assay coefficients of variation were 3.7% and 2.7%, respectively, and assay

sensitivity was 0.01 ng/mL. Recoveries after a 10% dilution of samples and mixing with high and low AMH concentrations ranged from 94% to 103%. The clinical cut-off for normal serum AMH was set at 1 ng/mL.

Analyses were conducted with SPSS version 19.0 (IBM, Armonk, NY, USA). Descriptive statistics were used to report data. Continuous variables were compared by using the unpaired Student *t* or Mann–Whitney *U* test; categoric variables were compared by using χ^2 test. Unconditional multiple logistic regression was used to explore the relationship between the presence of endometriosis and AGD (below/above median value) or AMH (below/above clinical cut-off) using odds ratios (ORs) and 95% confidence intervals (CIs). Age, height, weight, BMI, vaginal delivery, and episiotomy were tested as covariates. Vaginal delivery was included as a covariate because it might distort AGD in women; BMI was included because AGD is an anthropometry-dependent measure.

To assess the ability of AGD and AMH to detect endometriosis, receiver operator characteristic (ROC) curves were generated by using maximum likelihood estimation to fit a binomial ROC curve to continuously distributed data. The ROC curves were based on AGD_{AF} measurements, AMH levels, or both variables combined (AMH and AGD_{AF}), and the presence of all endometriosis or endometriomas versus controls. AGD measurements were dichotomized by using the optimal cut-off point based on the maximum Youden index, and sensitivity, specificity, and positive and negative likelihood ratios were calculated. All statistical tests were two-tailed at a 0.05 significance level.

3. RESULTS

During the study period, 57 women with endometriosis (45 with endometriomas and 12 with DIE), and 93 women without this condition were recruited. The women were aged 18–50 years. Overall, women with endometriosis were older (mean±SD age 36.2±7.6 vs 29.7±5.9 years; *P*<0.001) and had a higher incidence of vaginal delivery (22 [39%] vs 20 [22%]; *P*=0.010) as compared with the control group, but BMI and incidence of episiotomy were comparable between the two groups (Table 1). Women with endometriosis had significantly shorter AGD_{AF} (22.8±4.6 vs 27.2±5.7 mm; *P*<0.001) and lower AMH levels (2.2±2.5 vs 3.3±1.9 ng/mL; *P*=0.003). The subgroup of women with endometriomas presented similar descriptive characteristics. The subgroup of women with DIE was not evaluated separately owing to the small sample (n=12).

Women with serum AMH below the clinical cut-off (1 ng/mL) were 17.40 (95% CI 5.64–53.82) times more likely to have endometriosis. After controlling for important confounders, the association remained strong for both endometriosis (OR 13.32, 95% CI 3.31–53.52) and endometriomas (OR 14.82, 95% CI 3.51–63.12) (Table 2).

An AGD_{AF} measurement below the median value was also associated with endometriosis (Table 3). After adjustment for confounders, women with AGD_{AF} below the median were 3.72 (95% CI 1.64–8.50) and 2.82 (95% CI 1.23–6.64) times more likely to have endometriosis or endometriomas, respectively, as compared with women with a value above the median.

The accuracy of AMH, AGD_{AF}, and combined AMH and AGD_{AF} to diagnose endometriosis or endometriomas is summarized in Table 4. Overall, the area under the curve (AUC) was greater for AMH plus AGD_{AF} than for AMH or AGD_{AF} alone, indicating that AMH plus AGD_{AF} has a stronger predictive value for discriminating both endometriosis overall and the endometriomas subtype.

For endometriosis (n=57) versus controls (n=93), the AUC of AMH + AGD_{AF} was 0.77 (95% CI 0.70–0.85). The sensitivity and specificity of the model were 53% and 90%, respectively, and the positive and negative likelihood ratios were 5.30 and 0.52, respectively. For the endometriomas subgroup (n=45) versus controls (n=93), the AUC of AMH + AGD_{AF} was 0.73 (95% CI 0.64–0.82). The sensitivity and specificity of this model were 44% and 93%, respectively, and the positive and negative likelihood ratios were 6.29 and 0.60, respectively (Figure 1).

4. DISCUSSION

The present findings indicate that low levels of AMH (<1 ng/mL) might be used to identify women with endometriosis. Similarly, shorter AGD_{AF}, a biomarker of the intrauterine hormonal environment, might also predict the presence of this condition. To our knowledge, this is the first time that the combined ability of AMH and AGD_{AF} to predict endometriosis has been explored. The diagnostic accuracy of the combined predictive model was better than that of AMH or AGD alone.

Regarding AMH, the present findings are consistent with those of Pacchiarotti et al. [11], who proposed AMH as a biomarker capable of identifying ovarian depletion caused by early ovarian damage in progressive diseases such as endometriosis for

women who have not undergone surgery. Other studies have also suggested that endometriomas are associated with a decreased ovarian reserve, which results in lower AMH levels. Chen et al. [17] reported lower mean AMH for women with endometriomas (1.53 ± 1.37 ± ng/mL) than for women with benign ovarian cysts (2.20 ± 1.23 ng/mL). Moreover, the extent of AMH decline was greater for bilateral endometriomas than for unilateral ones, and no decrease was observed for women in the benign ovarian cyst group compared to endometriosis. Hwu et al. [18] also reported a decrease in AMH among women with endometriomas greater than 3 cm who had not undergone surgery, suggesting a significant reduction of ovarian reserve among these women even before cystectomy.

Kim et al. [10] described lower preoperative serum AMH levels among women with advanced ovarian endometriomas (stage IV), although they did not observe a significant reduction for other stages of endometrioma. By contrast, others found lower serum AMH levels among women with endometriosis after surgery, but could not confirm diminished ovarian reserve [9]. In addition, Carrarelli et al. [12] reported increased expression of the AMH receptor II in endometrium and endometriotic lesions with normal serum AMH levels among women with endometriosis, suggesting a local action of endometrial AMH and its possible involvement in the pathology of this disorder. Their findings also imply a limitation in using serum AMH levels as a marker of endometriosis. Lastly, Signorile et al. [19] evaluated in vitro the potential effects of AMH treatment on endometriotic lesions, reporting a decreased in cell viability and a higher percentage of cell death among endometriotic cells, and suggesting the potential use of AMH as a therapeutic agent in endometriosis.

With regard to AGD, a shorter AGD was recently reported among women with endometriosis, suggesting that these women have been affected by prenatal exposure to an antiandrogenic or estrogenic environment [13]. In addition, AGD_{AF} (but not AGD_{AC}) was previously found to be associated with the presence of endometriomas, DIE, or both, and was proposed as an efficient tool in predicting the presence of DIE (AUC, 0.91; 95% CI, 0.84–0.97) [14]. The two studies on AGD enrolled women with and without previous surgery in contrast to the present study, which included only women without surgery to avoid the potential effect of surgery on serum AMH levels.

The two markers proposed in the present study, AMH and AGD, are both related to an intrauterine origin of endometriosis; however, there are various hypotheses on the pathogenesis of this disorder. During organogenesis, genes from the Homeobox and Wingless family seem to be involved in differentiation of the ducts of Müller and in development of the urogenital tract [20,21]. Laganà et al. [20] proposed that deregulation of many genes involved in the differentiation and development of the urogenital tract and the Wnt signaling pathway (Wnt/β-catenin) leads to aberrations and deregulation within the mesoderm, and may cause aberrant placement of stem cells, leading to ectopic endometrial cells, in endometriosis [22].

Several predictive models for the diagnosis of endometriosis have been proposed, but most apply to a specific type of endometriosis, particularly DIE. For example, Chapron et al. [6] described a model based on symptoms and clinical history to diagnose DIE among women with chronic pelvic pain (sensitivity, 74.5%; specificity, 68.7%). Koninckx et al. [23] combined clinical markers with plasma CA-125,

reporting that levels higher than 35 U/mL were useful in deciding whether a bowel preparation (for suspected DIE) should be administered (sensitivity, 87%; specificity, 83%). Lafay Pillet et al. [24] described a model based on a clinical score of four variables independently associated with DIE that was used to classify patients as being in a low-risk group (score <13), where the probability of DIE was 10% (sensitivity of 95%), or a high-risk group (score>13), where the probability of DIE was 88% (specificity of 94%). Perelló et al. [7] have described a predictive model for DIE in patients with endometriomas based on one previous pregnancy, past history of surgery for endometriosis, and the mean endometriosis-associated pelvic pain score. The AUC was 0.91 (95% CI 0.86 to 0.94), the sensitivity of the model was 80%, and the specificity was 84%. Nnoaham et al. [25] produced a model that predicted stage III and IV endometriosis with a sensitivity of 82.3% and specificity of 75.8% based on menstrual dyschezia and a history of benign ovarian cysts. In a study by Eskenazi et al. [26], ovarian endometriosis was accurately predicted by signs, symptoms (infertility, dysmenorrhea, dyspareunia and noncyclic pelvic pain), and TVUS, with excellent agreement with surgical diagnosis. However, only 38% of non-ovarian endometriosis could be predicted using this model. Lastly, Nisenblat et al. [5] reported that TVUS showed relatively high sensitivity (93%) and specificity (96%) in the diagnosis of endometriomas.

The present study has some limitations. First, selection and measurement bias should be considered. However, the control women attended the study hospital in the same period, and were drawn from the same population as the women with endometriosis. Moreover, two gynecologists who were not from the endometriosis unit took the measurements and were unaware of the women's condition. Second,

endometriosis was not histologically confirmed; as mentioned above, however, TVUS has relatively high sensitivity (93%) and specificity (96%) for endometriomas [5] and can be used to assess disease status. Third, owing to the clinical diagnosis used for the current study, misclassification of disease status might have occurred. If present, however, it would have underestimated the value of the true association between AGD and endometriosis. Last, the number of patients was relatively small, potentially limiting the robustness of the results.

In conclusion, the present study has shown that serum AMH levels and AGD_{AF} can be combined in a model to predict endometriosis. As compared with others, this model has advantages because it applies to all types of endometriosis (rather than specific subtypes) among women who have not undergone surgery. In addition, it does not require previous laparoscopy or TVUS. This might help improve early diagnosis of endometriosis, which in turn would improve patient response to medical or surgical treatments [27]. Because the two markers are obtained by non-invasive techniques, they might be used by general practitioners in the diagnosis of endometriosis. More studies are needed to corroborate the present findings; if confirmed, the combination of AMH levels and AGD might have further clinical implications in the early diagnosis of endometriosis, thereby improving its prognosis.

Author contributions

MLS-F contributed to the conception and design of the study, data collection, data analysis and interpretation, and writing the manuscript. RJ-V, LC-L, AC-B, SC-B, and AIH-P contributed to the data collection and revising the manuscript. JM contributed to the conception and design of the study, data analysis and interpretation, and

writing the manuscript. MTP-S contributed to the data collection, data analysis and interpretation, and writing the manuscript. EA contributed to data analysis and interpretation, and writing the manuscript. AN contributed to the conception and design of the study, and revising the manuscript. AMT-C contributed to the conception and design of the study, and writing the manuscript. All authors contributed intellectually and approved the final version of the manuscript.

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Conflicts of interest

The authors have no conflicts of interest.

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Figure 1 ROC curves for AGD and presence of endometriosis. These analyses assess the ability of AGD to predict all endometriosis and endometriomas. Blue, green, and yellow unbroken lines represent AMH, AGD_{AF}, and AMH plus AGD_{AF}, respectively. (A) Presence of all endometriosis (n=57) versus control group (n=93); (B) presence of endometriomas (n=45) versus control group (n=93). Abbreviation: AGD, anogenital distance; AGD_{AF}, anogenital distance from the posterior fourchette to the upper verge of the anus; AMH, anti-Müllerian hormone; ROC, receiver operating characteristic.

Figure S1 Measurement of anogenital distance (AGD). Two measurements were made: AGD_{AC}, from the anterior clitoral surface to the center of the anus (left); and AGD_{AF}, from the posterior fourchette to the center (right).

Table 1 Descriptive characteristics of the women by study group [a].

Characteristic	Controls (n=93)	All endometriosis (n=57)	*P* value [b]	Endometriomas (n=45)	*P* value [b]
Age, y	29.7 ± 5.9 (31.0)	36.2 ± 7.6 (37.0)	<0.001	35.7 ± 7.9 (36.5)	0.001
BMI	23.6 ± 5.3 (22.0)	23.4 ± 3.5 (22.6)	0.490	23.0 ± 3.7 (22.0)	0.980
AMH, ng/mL	3.3 ± 1.9 (2.9)	2.2 ± 2.5 (1.3)	0.003	2.3 ± 2.6 (1.4)	0.010
AGD_{AF}, mm	27.2 ± 5.7 (26.1)	22.8 ± 4.6 (21.7)	<0.001	23.9 ± 4.3 (22.8)	0.001
Vaginal delivery	20 (22.0)	22 (39.0)	0.010	19 (42.2)	0.007
Episiotomy	16 (17.0)	6 (11.0)	0.260	7 (15.6)	0.730

Abbreviations: AGD_{AF}: anogenital distance from the posterior fourchette to the upper verge of the anus; AMH: Anti-Müllerian hormone; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).
[a] Values are given as mean ± SD (median) or number (percentage) unless stated otherwise.
[b] Versus control women by Student *t*, Mann–Whitney *U*, or χ^2 test.

Table 2 Odds ratio of endometriosis or endometriomas stratified by serum AMH cut-off for clinical assessment.

Serum AMH level	No. of patients	No. of controls	OR (95% CI)	*P* value	aOR (95% CI) [a]	*P* value
All endometriosis	57	93				<0.001
>1 ng/mL	32	89	1.0 (reference)		1.0 (reference)	
≤1 ng/mL	25	4	17.40 (5.64–53.82)	<0.001	13.32 (3.31–53.52)	
Endometriomas	45	93				<0.001
>1 ng/mL	26	89	1.0 (reference)		1.0 (reference)	
≤1 ng/mL	19	4	16.30 (5.10–52.04)	<0.001	14.82 (3.51–63.12)	

Abbreviations: AMH, anti-Müllerian hormone; aOR, adjusted odds ratio; CI, confidence interval; OR, odds ratio.
[a] Adjusted for age.

Table 3 Odds ratio of endometriosis or endometriomas stratified by median of AGD_{AF} measure for clinical assessment.

AGD_{AF} measurement	No. of cases	No. of controls	OR (95% CI)	*P* value	aOR (95% CI)	*P* value
All endometriosis	57	93				0.002
≥24.85 mm	19	56	1.0 (reference)		1.0 (reference)	
<24.85 mm	38	37	3.03 (1.52–6.03)	0.001	3.72 (1.64–8.50)	
Endometriomas	45	93				0.020
≥24.85 mm	18	56	1.0 (reference)		1.0 (reference)	
<24.85 mm	27	37	2.27 (1.10–4.70)	0.030	2.82 (1.23–6.64)	

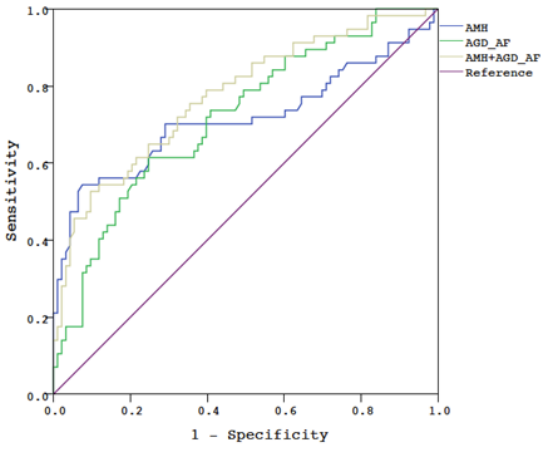
Abbreviations: AGD_{AF}, anogenital distance from the upper verge of the anus to the posterior fourchette; aOR, adjusted odds ratio; BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters); CI, confidence interval; OR, odds ratio.
[a] Adjusted for age, BMI, and vaginal delivery.

Table 4 Diagnostic accuracy of AMH, AGD_{AF}, and their combination for endometriosis or endometriomas [a].

Variable	Control group	Endometriosis group	*P* value [b]	AUC (95% CI)	Diagnostic accuracy				
					Cutoff [c]	Sensitivity, %	Specificity, %	+LR	–LR
All endometriosis	93	57							
AMH, ng/mL	3.3 ± 1.9	2.2 ± 2.5	0.010	0.71 (0.62–0.81)	≤1.45	54.0	93.0	7.71	0.49
AGD_{AF}, mm	27.2 ± 5.7	22.8 ± 4.6	0.008	0.72 (0.64–0.80)	≤22.8	60.0	76.0	2.50	0.53
AMH+AGD_{AF} [d]	0.74 ± 0.18	0.45 ± 0.22	0.004	0.77 (0.70–0.85)	≤0.46	53.0	90.0	5.30	0.52
Endometriomas	93	45							
AMH, ng/mL	3.3 ± 1.9	2.3 ± 2.6	0.010	0.70 (0.59–0.81)	≤1.45	51.0	93.0	7.29	0.53
AGD_{AF}, mm	27.2 ± 5.7	23.9 ± 4.3	0.007	0.67 (0.57–0.76)	≤25.4	69.0	60.0	1.73	0.52
AMH+AGD_{AF} [d]	0.75 ± 0.15	0.50 ± 0.19	0.006	0.73 (0.64–0.82)	≤0.51	44.0	93.0	6.29	0.60

Abbreviations: AGD_{AF}, anogenital distance from the upper verge of the anus to the posterior fourchette; AMH, anti-Müllerian hormone; AUC, area under the receiver operating characteristic (ROC) curve; LR, likelihood ratio.
[a] Values are given as absolute number or mean ± SD unless stated otherwise.
[b] Versus control women by Student *t* test.
[c] Dichotomized by Youden index (maximum potential effectiveness for sensitivity and specificity) [28].
[d] Binary logistic regression was used to compute predicted values (probabilities) of the combined variables; the ROC curve was then generated from these values.

A



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