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# Can endometrial arylsulfatase A activity predict the onset of endometrial polyps over the years?

*A atividade da arilsulfatase endometrial A pode prever a aparição de pólipos endometriais ao longo dos anos?*

## Artigo Original

### Keywords

Arylsulphatase A  
Polyps  
Endometrium  
Gonadal steroid hormones  
Sulphatides  
Endometrium

### Palavras-chave

Cerebrosídeo sulfatase  
Pólipos  
Endométrio  
Hormônios esteroides gonadais  
Sulfatidas  
Endométrio

### Abstract

**PURPOSE:** To assess if arylsulfatase A activity (ASA) and sulfatide (SL) concentration in the human endometrium can be predictive of the development of endometrial polyps over the years, since ASA activity reflects the endometrial sensitivity to hormones. **METHODS:** ASA activity and SL concentration were determined by biochemical procedures on endometrial samples collected between 1990 and 1994 in non-menopausal women. These women underwent a new endometrial sampling following the clinical indication some years after the first endometrial sampling. The histological assessment of the second endometrial specimens found four patients with normal endometrial pattern and 10 patients with one or more endometrial polyps. ASA activity/years elapsed and SL concentration/years elapsed were compared using two tailed Mann-Whitney test for unpaired data between patients with normal pattern and patients with endometrial polyps. **RESULTS:** Median ASA activities were 2.62 (normal pattern) versus 1.85 (endometrial polyps) nmol hydrolyzed substrate/min. Median activity/years elapsed is higher in patients with second endometrial sample presenting normal pattern ( $p=0.006$ ) and median SL concentration/years elapsed does not differ significantly among groups, even if median SL concentration seems to be higher in patients who subsequently developed polyps (1031  $\mu\text{g/g}$  of fresh tissue versus 341,5  $\mu\text{g/g}$  of fresh tissue). **CONCLUSIONS:** ASA activity can predict the onset of endometrial polyps over the years.

### Resumo

**OBJETIVO:** Avaliar se a atividade da arilsulfatase A (ASA) e a concentração de sulfatida (SL) no endométrio humano pode ser preditivo em relação ao desenvolvimento de pólipos endometriais ao longo dos anos, posto que atividade da ASA reflete a sensibilidade do endométrio aos hormônios. **MÉTODOS:** A atividade da ASA, assim como a concentração de SL, foi determinada por meio de procedimentos bioquímicos em amostras de endométrio coletadas entre 1990 e 1994, em mulheres que não se encontravam na menopausa. Essas mulheres foram submetidas a uma nova amostragem endometrial após indicação clínica alguns anos depois da primeira amostragem endometrial. A avaliação histológica dos segundos espécimes endometriais permitiu identificar quatro pacientes com padrão endometrial normal e 10 com um ou mais pólipos endometriais. A atividade da ASA/anos depois e a concentração de SL/anos depois foram comparadas, utilizando o teste bilateral U de Mann-Whitney para dados não pareados entre as pacientes com padrão normal e as pacientes com pólipos endometriais. **RESULTADOS:** A atividade da ASA foi 2,62 (padrão normal) em comparação com 1,85 (endometrial pólipos) de substrato hidrolisado/min. A atividade da ASA/anos depois é maior em pacientes com segunda amostra endometrial a apresentarem um padrão normal ( $p=0,006$ ), e a concentração mediana de SL/anos depois não difere de forma significativa entre os grupos, apesar de a concentração mediana de SL parecer maior em pacientes que posteriormente desenvolveram pólipos (1031  $\mu\text{g/g}$  de tecido fresco em comparação com 341,5  $\mu\text{g/g}$  de tecido fresco). **CONCLUSÕES:** A atividade da ASA pode prever a aparição de pólipos endometriais ao longo dos anos.

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Results were found from samples collected at the Obstetrics School of Camerino. This institution closed in 1997, therefore the address cannot be supplied.

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## Introduction

Endometrial behavior during the menstrual cycle is closely related to the expression of hormonal receptors in the glandular epithelium and stroma<sup>1</sup>. As opposed to patients with normal cycles, patients with dysfunctional bleeding have higher levels of estrogen receptors in the second phase of the cycle<sup>2</sup>, which suggests that their endometrium is more susceptible to estrogen stimulation.

It is thought that endometrial polyps may be related in some ways to hyperestrogenism both in premenopausal and postmenopausal patients<sup>3</sup>. Therefore, patients with dysfunctional bleeding, more sensitive to estrogens<sup>2</sup>, may develop endometrial polyps over the years in relation to endometrial estrogen sensitivity and to the duration of estrogen stimulation of endometrium.

Many genes are expressed in the endometrium during the menstrual cycle in relation to hormonal stimulation, with the goal to prepare the endometrium for implantation<sup>4</sup>. Arylsulphatase A (ASA) expression is regulated by estrogens and progesterone, because estrogens decrease ASA activity and progesterone increases it<sup>5-8</sup>. ASA is a lysosomal enzyme that catabolizes sulphatides (SL). To date, it is not known what role SL plays in the physiology of the endometrium.

The objective of this study is to evaluate whether ASA activity and SL concentration can predict the onset of endometrial polyps over the years, because hormonal control of ASA activity is a marker of endometrial hormonal sensitivity.

## Methods

Starting in 1990, a study on endometrial specimens collected at the Obstetrics School of Camerino (Italy) was initiated. The research aimed to assess ASA activity and SL concentration in normal endometrium through the endometrial cycle and in endometrial pathologies, and results were published in 1992 and 1994<sup>6,9</sup>. Such analyses were previously approved by local ethics committees. Among samples collected between 1990 and 1994, some cases were excluded from data published in 1992 and 1994<sup>6,9</sup> because patients did not meet the inclusion criteria. However, ASA activity and SL concentration were nonetheless assessed on such specimens.

The names and birth dates of the excluded non-menopausal patients and the names and birth dates of non-menopausal patients enrolled in previous studies<sup>6,9</sup> were transmitted to the Operative Unit of Pathology of the hospital of Macerata (Area Vasta 3 – 3 Marche). Here, it was observed whether or not some of these patients had undergone another endometrial biopsy, collected between 1995 and 2011. Therefore, this is a retrospective study. The search retrieved 14 cases, four of which had normal endometrium, and 10 of which had endometrial polyps.

ASA activity and SL concentration analyzed on endometrial specimens collected from 1990 and 1994 were compared in the group of patients with negative endometrium in the subsequent endometrial samples and in the group of patients who developed endometrial polyps. To control the effect of time (years) between the first and the second endometrial sample, ASA activity and SL concentration were divided by the years elapsed between the two samplings, to obtain an index for statistical analysis. Two tailed Mann-Whitney test for unpaired data was used for statistical analysis, with  $p < 0.05$  as the minimum for significance. Kypplot 2.0 was used for statistical analysis.

The specimens collected from 1990 to 1994 were obtained after dilatation and curettage (D&C). A portion of each sample was processed for histological diagnosis; the other portion was stored at  $-20^{\circ}\text{C}$ . Extraction and separation of sulfatides were carried out according to a method adapted from Bolognani et al.<sup>10</sup>: The samples were homogenized in chloroform/methanol (2:1 w/v) and the extract was filtered through a sintered glass with a vacuum pump connection. KCl (0.88%) was added to the filtrate and, after stirring, the covered tube was left until the separation phase (aqueous and organic phase) occurred. The organic phase was analyzed by means of a spectrophotometric procedure for sulfatides, using a 0.15 mm Azure A solution in 0.05 N H<sub>2</sub>SO<sub>4</sub> for staining<sup>11</sup>. Absorbance was measured at 640 nm against a standard containing 70 mg/g of sulfatides. Concentration was expressed as  $\mu\text{g/g}$  of fresh tissue.

The organic phase was also used for thin layer chromatography (TLC) separation on a 0.25 mm silica gel plate. Lipids were separated on the same plate using a solvent mixture of chloroform-methanol-water (70:30:5 per volume) and compared with a standard polar lipid mixture (phosphatidylcholine, sulfatides, phosphatidylethanolamine and cerebrosides). The plates were exposed to iodine vapours to visualize the lipid fractions in order to quantify sulfatide concentration.

To assess ASA activity, samples were homogenized for 2 minutes in double distilled water (1:20 w/v) containing 0.1% Triton *versus* 100 with Ultraturrax TP 18/10 homogenizer (Janke & Kunkel, Staufen, Germany). The homogenate was centrifuged at 20,000 rpm at  $4^{\circ}\text{C}$  for 30 minutes using a refrigerated centrifuge (IEC Centra SR, Dunstable, Bedfordshire, England). ASA activity was determined biochemically according to a method by Vitaioli et al.<sup>12</sup>, adapted from Percy and Brady<sup>13</sup>. The incubation mixture consisted of 0.01 M p-nitrocatechol sulfate in 0.5 mM sodium acetate buffer at pH 5.0 containing mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub>, and 10% w/v NaCl, and 10–50 mg of protein. The mixture was incubated at  $37^{\circ}\text{C}$  and the reaction stopped after 30 minutes by adding 1 N NaOH. The absorbance of a reaction product, p-nitrocatechol, was

measured at 515 nm against a blank using a Beckman DU 40 spectrophotometer (Irvine, USA). The specific activity was expressed as nmol hydrolysed substrate/min per mg protein, assuming  $14.0 \times 10^3$  as the molar extinction coefficient for the hydrolysis product, p-nitrocatechol, of the substrate at 515 nm. Protein concentrations were measured according to the Bradford's<sup>14</sup> method.

P-nitrocatechol sulfate and standard polar lipids mixture were purchased from Sigma Aldrich Chemical Co. (St. Louis, MO). All other chemicals were of analytical grade and were purchased from Serva (Heidelberg, Germany). The 0.25 mm precoated silica gel plates, used for TLC, were purchased from Merck Co. (Dormstadt, Germany).

Endometrial samples taken between 1995 and 2011 were obtained from hysteroscopic biopsies as a routine endometrial assessment for irregular bleeding or ultrasonographic suspicion of endometrial diseases within the entire health district of Area Vasta 3 – Marche.

## Results

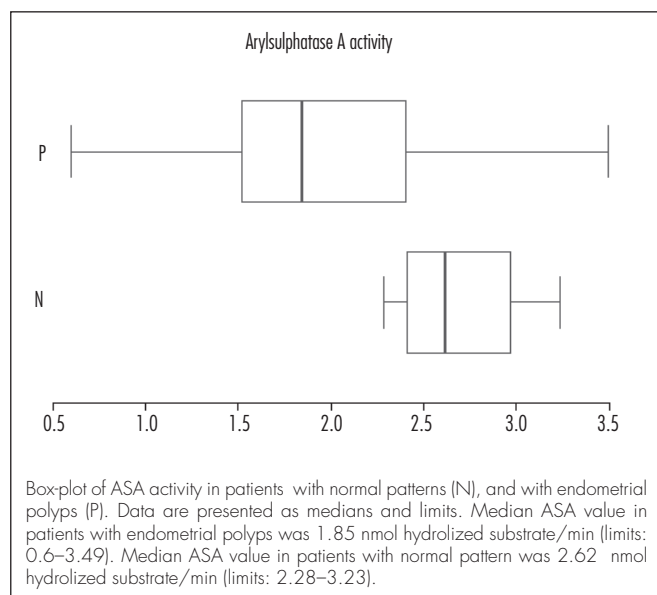
Table 1 summarizes histological diagnoses for the first and second endometrial specimens. Additionally, Table 1 reported the patient's age at the first endometrial sampling and the years elapsed from first sampling to second. ASA activities and SL concentrations reported in Table 1 are the mean of four determinations for each patient.

Median ASA activity in patients with the second endometrial sample resulting in normal pattern was 2.6 nmol hydrolyzed substrate/min (limits: 2.28–3.23). Median ASA activity in patients with the second endometrial sample diagnosed as endometrial polyp was 1.85 nmol hydrolyzed substrate/min (limits: 0.6–3.4). A box-plot describing ASA activities in both groups of patients is represented in Figure 1. After correction for years elapsed from the first and the second endometrial sampling, median ASA activity/years elapsed is higher in

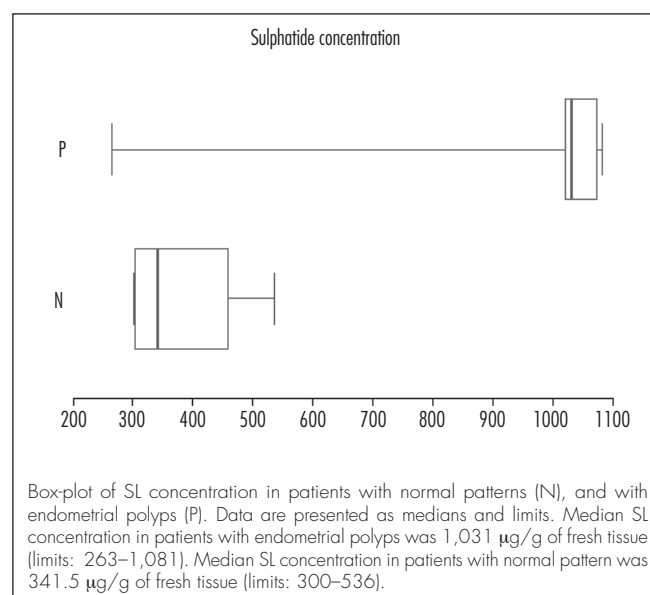
**Table 1.** Descriptive data

Age at time of first biopsy (median–limits)	Histological diagnoses (between 1990–1994) (rates)	ASA activity* (mean±SD)	SL concentration** (mean±SD)	Histological diagnoses (after 1994) (rates)	Years elapsed from first endometrial sampling to second (median–limits)
43.5 37–52	Simple non atypical hyperplasia 14.3%	2.19±0.76	760.14±355.01	Normal patterns 28.6%	6.5 4–15
	Dysfunctional endometrium 21.4%			Endometrial polyps 71.4%	
	Proliferative endometrium 28.6%				
	Secretive endometrium 7.1%				
	Endometrial polyps 21.4%				

\*ASA (Arylsulfatase A) activity is expressed as nmol hydrolysed substrate/min. \*\*SL (sulphatides) concentration is expressed as µg/g of fresh tissue. SD: standard deviation



**Figure 1.** Box-plot of Arylsulfatase A activity.



**Figure 2.** Box-plot of Sulphatide concentration.

patients with the second endometrial sampling resulting in normal pattern ( $p=0.006$ ).

Median SL concentration in patients with second endometrial sample resulting in normal pattern was 341.5  $\mu\text{g/g}$  of fresh tissue (limits: 300–536). Median SL concentration in patients with the second endometrial sample diagnosed with endometrial polyp was 1,031  $\mu\text{g/g}$  of fresh tissue (limits: 263–1081). A box-plot describing SL concentrations in both group of patients is represented in Figure 2. Median SL concentration/years elapsed does not differ significantly between groups, even if the mean concentration of SL seems to be greater in patients who subsequently developed polyps.

## Discussion

Hyperestrogenism as the cause of endometrial polyps is a common belief in the international literature<sup>3</sup>, even if the cause of the onset of endometrial polyp is still unknown<sup>15</sup>. It should be considered that tissue sensitivity to estrogens is related to the expression of estrogen receptors alpha and beta. As reported by Ye et al.<sup>16</sup>, the overexpression of estrogen receptor beta may lead to the formation of endometrial polyps.

The expression of estrogen receptors is modulated by endometrial pulsed stimulation of estrogens in post-menopause<sup>17</sup>. Moreover, aging alters estrogen sensitivity as well<sup>18-20</sup>. It is also reported<sup>16</sup> that estrogen receptor beta is directly related to estrogen levels in serum. Therefore, hyperestrogenism could lead to the onset of endometrial polyps acting on the relative expression of estrogen receptor beta over the years. We hypothesize that ASA activity is linked with the overall effect of estrogen receptors beta, alpha, and progesterone receptors. A relative abundance of estrogen receptors beta physiologically prevents the expression of estrogen receptor alpha and, consequently, of progesterone receptors<sup>21</sup>. Therefore, in patients that further develop endometrial polyps, a less intense activity of ASA should be found as a sign of less intense sensitivity to progesterone. The ASA activity reported in our data should be strictly related to estrogen receptor alpha/beta ratio. Therefore, ASA activity predicts the formation of endometrial polyps over the years, because a chronically irregular estrogen stimulation of the endometrium leads to the

formation of endometrial polyps<sup>3</sup>. After correcting the ASA activity values by years elapsed from the first to the second endometrial sampling, it is demonstrated that ASA activity predicts the onset of endometrial polyps.

Interestingly, the SL concentration does not seem to be related to the formation of endometrial polyps. As demonstrated in Figure 2, however, data are dispersed, leading to difficulties to interpret the results. However, a dispersion of SL concentrations in decidua and fetal adnexa was previously found<sup>22,23</sup>, thus suggesting that the turnover of SL may be related to other control mechanisms involving both biosynthesis and catabolism. Therefore, steroids may control the SL turnover in many unknown ways overall.

The major limitation of this study is the small sample of followed-up patients. Due to this small sample size, readers must be cautious to interpret the results as being conclusive. Moreover, the sample size may justify the lack of findings about the SL behaviour. We are not able to follow-up more patients, because the endometrial samples were collected with other goals, and, consequently, many patients were lost to follow-up. Therefore, readers should consider those findings as *post-hoc* results, suggesting further investigations about the behaviour of ASA in the pathophysiology of the human endometrium.

Additionally, this study does not assess steroid receptor expression in relation to ASA activity and SL concentration behavior. This is a limiting factor if one wants to support the hormonal hypothesis of ASA activity control. We were unable to check the steroid receptors expression in the endometrial specimens. However, previous data widely suggested that ASA activity is enhanced by progesterone<sup>5,6,22,23</sup>.

In conclusion, the results suggest that ASA activity may predict the formation of endometrial polyps over the years, because it is a marker of lack of endometrial progesterone sensitivity.

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