# Immunological evaluation of vaginal secretion in patients with high-grade cervical intraepithelial neoplasia treated with intralesional interferon α-2b

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**Summary**

*Introduction:* Conservative treatment with intralesional interferon (IFN) is a therapeutic option for cervical intraepithelial neoplasia (CIN) patients of childbearing age. *Materials and Methods:* The study group was made up of patients diagnosed with a high-grade lesion and treated with intralesional human recombinant IFNα-2b. Vaginal secretion was collected during IFNα-2b treatment for analysis of cytokines and viral load. *Results:* The initial histology diagnostic was 62.5% (n = 5) with CIN 2 and 37.5% (n = 3) with CIN 3. In terms of clinical evaluation and anatomopathology, 6.5% (n = 5) had a good clinical response, while 37.5% (n = 3) had therapeutic failure. All the patients with therapeutic failure were smokers. Interleukin 6 and tumor necrosis factor-α concentrations were raised at the sixth application for the patient group who failed to respond to therapy compared to the responsive group ($p = 0.0357$). Patients with a good response exhibited a reduction in human papillomavirus viral load ($p = 0.03$). *Conclusions:* Patients that had a good response had lower concentrations of inflammatory cytokines than did non-responders.

*Key words:* Human papillomavirus virus; Cervical intraepithelial neoplasia; Treatment; Interferon α-2b; Cytokines.

## Introduction

Cervical cancer is a world health problem. Globally, it is the second most common tumor among women [1]. It is preceded by pre-malignant lesions, called cervical intraepithelial neoplasias (CINs), which can take years to evolve [2]. The development of these lesions and, consequently, of cervical cancer is intimately associated with the human papillomavirus (HPV) infection [3].

Cervical HPV infection is temporary for the majority (70-90%) of women infected, with the virus being eliminated 12-24 months after initial diagnosis. However, persistent HPV, principally in its oncogenic forms, is closely associated with the development of high-grade cervical lesions [4]. The virus's persistence is closely linked to mechanisms that evade the host's immune response [5, 6].

Cell mediated immune response, at systemic and local levels, is important to the course of HPV infection [7]. Evidence shows that the interaction of cytokines liberated during cellular and humoral immune responses is responsible for the remission, persistence, and progression of HPV-related lesions, although the mechanisms involved in these responses are not yet well understood [8].

Currently, treatment of high-grade lesions by ablative (laser) or excisional (cold conization, laser conization, loop diathermy) methods is recommended, with the former only being advocated following a satisfactory colposcopy [9]. However, various studies have noted an increase in obstetric complications in women who had previously received these procedures, with the most common of these being pre-term delivery, low birth weight, and premature membrane rupture [10-13].

Given the rising number of people infected with HPV and the appearance of CIN in young people [14], alongside the fact that women are having children later and later in life, the need to develop therapies that do not interfere with patients' future reproductive capability has become pressing. Conservative treatment with intralesional interferon (IFN) could be a therapeutic option for women of childbearing age since it does not alter the cervix's anatomy, which is a major factor in the development of complications during pregnancy.

Since the 1980s, various studies have used IFN for treating gynecological cancers with varying results [15]. With IFN treatment, studies have found remission of CINs in 30-80% of the cases [16-18]. With respect to invasive neoplasia, there are reports of curing invasive vaginal carcinoma using intralesional IFNα-2b [19].

Knowledge of the immunological changes that occur with interferon therapy is of fundamental importance in the development of strategies to treat cancer and its precursor lesions. To date, no study has evaluated the immunological changes in vaginal secretion caused by the intralesional use of IFN and the relationship these changes have with clinical response to treatment. The aim of this study was to analyze the concentration of cytokines in vaginal secretion, before and after treatment with intralesional IFNα-2b.

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## Materials and Methods

*Patients*

A prospective study was performed at the Oncology Research Institute (Instituto de Pesquisa em Oncologia, IPON) at the Federal University of the Triângulo Mineiro. The study group consisted of patients between 18 and 50 years of age with a diagnosis of CIN grades 2 and 3 who had not received prior treatment. Information about age, habits, and lifestyle (smoking, drug use, number of partners), and contraceptive methods used was gathered. Patients were advised to use condoms during their entire course of treatment. Patient identification was by number, with the first patient to participate in the study being labeled "1", the second "2", and so on.

The exclusion criteria were: immunosuppressant illness, serious cardiopathies or change in liver or kidney function; pregnancy; use of anti-inflammatories or immunosuppressants that could not be suspended during IFN treatment; reported intolerance of interferon; absence of a lesion visible by colposcopy or a very small lesion (diameter < 1 $cm^2$).

The Research Ethics Committee of the Federal University of the Triângulo Mineiro approved the study. All patients or their family members signed a free and clear consent form in writing.

*Colposcopy and biopsies*

The selected patients already had a biopsy that was positive for a high-grade CIN. Before the first and last treatment application, patients received a colposcopic examination; the images were photographed using a videocolposcope (Video Diagnose "Software" Program).

During the final application of interferon, a biopsy and a triple screen pap smear were performed. The biopsy was sent for colposcopy with the aid of a 24 cm, 2 mm Thomas Gaylor forceps. The fragment was embedded in a formaldehyde solution for anatomopathological study to confirm response to treatment.

*IFN application*

This study used 3,000,000 U of human recombinant IFN -2b (Blauferon B Blausiegel). The applications were made using a 1.0 ml syringe and a 13 × 0.45 needle three times a week on alternate days (Mondays, Wednesdays, and Fridays) for six consecutive weeks, making a total of 18 applications. Each application administered a dose of 3,000,000 U.

The cervix was exposed using a vaginal speculum, and antisepsis of the cervix and vaginal walls involved gauze soaked in topical povidone, using a *Cherron* forceps. The medicine was then applied. In the case of multiple lesions or those occupying more than one quadrant of the cervix, alternate applications were made on each lesion (in the case of isolated lesions) or in each quadrant (in the case of continuous lesions). During treatment, lactate dehydrogenase, hemoglobin, leukocytes, plaques, alkaline phosphatase, prothombin time, and activated partial thromboplastin time, urea, creatine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured, since IFN can influence these factors.

*Collection of vaginal secretion for cytokine dosage*

Vaginal secretion was collected by introducing a cytology brush. After being introduced, the brush was rotated once at the bottom of the vaginal pocket. This was then placed in a 0.5 ml *Eppendorf* tube containing 0.3 ml of saline solution. After breaking the stem, the seal was closed, and the tube was shaken on a *vortex* for 1 min, turned upside down, and a hole was made in the lower part of the tube. The contents were carefully transferred to a 1.5 ml Eppendorf tube and subjected to centrifugation for 500 min at 300 *g*. After centrifugation, the first tube was thrown out, and the material was finally stored at –20°C for later measurement. Vaginal secretion was collected after the first, sixth, tenth, and eighteenth (final) application.

*Flow cytometry*

Detection of the IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, tumor necrosis factor (TNF), INF-γ molecules was by flux cytometry (*Cytometric bead array*) in a BD FACS Calibur TM flux cytometer, in accordance with the Becton Dickinson (BD CBA) protocol.

The BD CBA protocol uses the sensitivity of amplified immunofluorescence detection through cytometry to measure elements based on immunoassay and allows for multiple detections within a small volume. Human Inflammation Kits (IL-8, IL-1β, IL-6, IL-10, TNF, IL-12) and the Th1/Th2 Kits (IL-2, IL-4, IL-6 IL-10, TNF, IFN-γ) were provided by Becton & Dickson (BD). The cytokines captured were incubated with antibodies and formed complex "sandwiches" that work in the BDA CBA, and the results were obtained in the form of graphs (as shown in Figures 1 and 2). Concentrations were obtained using the BD CBA software. The entire methodology was based on the product catalog.

*Evaluation of high-risk HPV viral load*

The hybrid capture technique was used for the HPV research. The entire collection procedure was performed according to the guidance of Digene of Brazil, which furnished the *kits* and equipment used in the hybrid capture test. The Captura Híbrida II System DML 2000 brand microplaque system with signal amplification was used for chemiluminescence. The information and methodology described below conform to the instructions provided by Digene of Brazil. Samples were collected from all patients prior to the start of treatment and at the conclusion of the treatment.

*Evaluation of clinical response*

For clinical response criteria after biopsy, complete disappearance of a high-grade lesion, that is reduction of moderate (CIN 2) or severe (CIN 3) dysplasia to mild (CIN 1) dysplasia or no apparent lesion as confirmed by histological study, was considered a response. Therapy was considered to have failed when a high-grade lesion persisted or progressed. All patients who showed persistence of a high-grade lesion (CIN 2 or CIN 3) were immediately taken for complementary surgical treatment (loop diathermy or conization).

*Statistical analysis*

Statistical analysis was performed using the GraphPad Prism 4 program and Microsoft Excel. Cytokine levels in each group of patients (responsive vs failed) were analyzed by the Friedman test, followed by Dunn's test, to define the differences between samples (initial sample, $6^{th}$ application, $12^{th}$ application, and $18^{th}$ application). The Mann-Whitney test was used to analyze differences between responses and failures in each sample. Wilcoxon's test was used to compare viral load before and after treatment in the two groups. Statistical significance was established at $p < 0.05$.

## Results

Eight patients participated in this study, with a minimum age of 22 years, a maximum age of 50 years, and a median age of 31.5 years. In terms of the habits and lifestyles queried in the initial protocol (parity, smoking, and number of sexual partners), 75% (n = 6) of the patients were multiparous, 62.5% (n = 5) were smokers, 62.5% had a history of three or more sexual partners, and the average age of sexarche was 16.75 years (minimum 14, maximum 18).

Initially, 62.5% (n = 5) of the patients were diagnosed with CIN 2 and 37.5% (n = 3) were diagnosed with CIN 3 (Table 1). Colposcopic findings of large lesions were observed in 100% of the patients; these lesions consisted of dense acetowhite epithelium (100%, n = 8), an irregular mosaicism (50%, n = 4), irregular punctation (62.5%, n = 5), and atypical vessels (37.5%, n = 3). In patients with a good clinical response (62.5%), colposcopic analysis showed signs of lesion regression by the second week after commencement of the injections. The following resolution could be observed: fragmentation of the mosaic and graduated attenuation of the areas of dense acetowhite epithelium with their substitution by metaplastic squamous epithelium.

When clinical response was evaluated, 62.5% (n = 5) of the patients showed a good response, while 37.5 (n = 3) showed therapeutic failure. All three patients with therapeutic failure were smokers. In terms of the lesion size before treatment, 25% (n = 2) had lesions occupying only one quadrant of the cervix, and 75% (n = 6) had lesions occupying more than one quadrant. Comparing lesion size to clinical response, patients with lesions occupying only one quadrant (n = 2) had a 100% response rate to treatment, whereas patients with a lesion occupying more than one quadrant (n = 6) had a 50% (n = 3) response rate and a 50% (n = 3) rate of therapeutic failure. Thus all patients with therapeutic failure had a lesion occupying more than one quadrant.

We observed a significant drop in HPV viral load in patients who responded to treatment ($p = 0.0313$ vs before treatment, Wilcoxon's test) (Table 2). On the contrary, patients in whom the treatment failed showed an increase in viral load after IFN treatment.

All eight (100%) of the patients experienced secondary effects from the medication, including myalgia, low fever (~38 ºC), and asthenia. These symptoms were confined to the day of application, beginning, on average, two hours after application and lasting for up to eight hours. In no case was it necessary to interrupt or suspend treatment. No patient showed altered liver, kidney, or coagulation function, and the examinations performed during treatment (lactate dehydrogenase, hemoglobin, leukocytes, plaques, alkaline phosphatase, prothombin activation time, and activated partial thromboplastin time, urea, creatine, AST and ALT) showed no changes with treatment.

Table 1 shows the initial and final diagnosis (before and after treatment) of each patient, as well as their clinical response to treatment and the actions taken in each case. Patients with good response were sent for trimesterly tracking by the colposcopy outpatient service, while of those who experienced treatment failure (n = 3), one underwent loop diathermy and two conization (with the treatment indicated based principally on the size of the lesion).

Table 1. — *Initial and final diagnosis by biopsy of all patients and the actions taken in each case (after IFN treatment).*

| Patient | Age (years) | Initial diagnosis | Final diagnosis | IFN treatment outcome | Action |
|---|---|---|---|---|---|
| 1 | 23 | CIN 2 | CIN 1 | Response | Tracking |
| 2 | 22 | CIN 3 | CIN 1 | Response | Tracking |
| 3 | 36 | CIN 3 | HPV infection | Response | Tracking |
| 4 | 50 | CIN 2 | CIN 1 | Response | Tracking |
| 5 | 25 | CIN 2 | CIN 3 | Failure | Loop diathermy |
| 6 | 30 | CIN 2 | HPV infection | Response | Tracking |
| 7 | 38 | CIN 3 | CIN 2 | Failure | Conization |
| 8 | 28 | CIN 2 | CIN 2 | Failure | Conization |

Table 2. — *Viral load of patient groups before and after treatment.*

| Patient | Viral load (mean/standard deviation) | |
|---|---|---|
| | Before treatment | After treatment |
| Failure | 6324/5213 | 84116/83683 |
| Response | 15879/9661 | 1580/1207* |

*($p = 0.0313$ vs before treatment, Wilcoxon's test).

Table 3. — *Concentrations of cytokines during treatment for each group of patients.*

| Cytokine | Patient Group | Cytokine level (mean/standard deviation) | | | |
|---|---|---|---|---|---|
| | | 1st applic. | 6th applic. | 12th applic. | 18th applic. |
| IFN-γ | Failure | 4.533/4.533 | 6.767/0.448 | 7.167/6.147 | 0.833/0.833 |
| | Response | 2.580 /2.027 | 2.420/0.872 | 3.440/0.642 | 3.460/2.038 |
| IL-10 | Failure | 12.93/12.29 | 6.900/3.148 | 4.767/2.347 | 2.833/0.589 |
| | Response | 3.080/0.421 | 2.420/0.765 | 3.020/0.354 | 3.700/0.272 |
| IL-6* | Failure | 583.3/434.7 | 2010/289.8 | 887.6/460.0 | 274.5/175.0 |
| | Response | 59.50/18.05 | 179.5/95.39 | 483.7/201.1 | 51.44/38.30 |
| IL-4 | Failure | 2.700/1.041 | 3.400/0.264 | 3.467/0.523 | 2.433/0.545 |
| | Response | 3.160/0.597 | 2.780/0.251 | 3.160/0.240 | 2.180/0.556 |
| IL-2 | Failure | 4.600/1.474 | 5.733/0.145 | 4.267/0.952 | 3.700/0.305 |
| | Response | 4.520/0.891 | 4.700/0.843 | 5.480/0.432 | 4.400/0.555 |
| IL-1β | Failure | 4131/4050 | 1690/1655 | 38440/38305 | 432.7/214.7 |
| | Response | 75.62/48.54 | 56.94/31.05 | 132.0/94.07 | 23.64/17.13 |
| IL-12 | Failure | 1.667/0.176 | 1.200/0.600 | 2.167/0.968 | 1.000/0.500 |
| | Response | 2.640/1.710 | 1.180/0.330 | 1.480/0.086 | 1.740/0.231 |
| IL-8 | Failure | 3605/1395 | 5636/636.1 | 5330/329.7 | 3354/1646 |
| | Response | 10489/3936 | 22893/17892 | 3303/895.2 | 5459/2455 |
| TNF-α* | Failure | 2.333/0.835 | 13.20/10.45 | 3.400/2.007 | 1.267/0.636 |
| | Responses | 2.780/1.664 | 1.380/0.394 | 1.540/0.060 | 1.880/0.086 |

*$p = 0.0357$, Mann-Whitney test, showing significant elevation in the sixth application in the group of patients with therapeutic failure vs the group with a good response; applic., application.

Table 3 shows the results of the cytokines analyzed in the vaginal secretion samples. In women with therapeutic failure, there was an increase in the average concentration of IFN-γ over the first three treatments and a decline at the time of the final application ($p = 0.5243$, Friedman test). In those with good response, the levels were essentially constant throughout the treatment ($p = 0.6522$, Friedman test). The patients with therapeutic

failure had a more elevated average concentration of IL-10 in the first sample than patients who responded to treatment ($p = 0.5714$, Mann-Whitney test). During treatment, patients with failure had falling levels of this IL-10 ($p = 0.7274$, Friedman test), while those who responded maintained essentially constant levels ($p = 0.5610$, Friedman test). The average concentration of IL-6 was significantly higher at the sixth application in the patients with failure than in those that responded ($p = 0.0357$, Mann-Whitney test). The average concentration of IL-4 stayed essentially constant in the patients with therapeutic failure ($p = 0.9097$, Friedman test) and in the good responders ($p = 0.1066$); a similar pattern was also observed with IL-2. Patients with therapeutic failure had very high concentrations of IL-1β throughout treatment ($p = 0.7274$, Friedman test), while among those who responded, IL-1β concentrations stayed lower and virtually flat (p = 0.5206, Friedman test). With respect to IL-12, no significant variation in concentrations were observed in either the patients whose treatments failed ($p = 0.6076$, Friedman test) or the good responders ($p = 0.8566$, Friedman test). A stable average IL-8 concentration was observed in patients with treatment failure ($p = 0.3420$, Friedman test) and falling levels were observed in the good responders ($p = 0.6522$, Friedman test). At the sixth application, the average concentration of TNF-α was significantly higher for the group with therapeutic failure than for group that responded well ($p = 0.0357$, Mann-Whitney test).

## Discussion

Evidence shows that various factors increase the risk of HPV infection and the development of CIN or cancer, among them smoking, parity [20], the number of sexual partners and sexual activity before 25 years of age [21]. In our study, all of these factors were observed: 75% were multiparous (3 or more births), 62.5% of the patients were smokers, 62.5% had a history of three or more partners, and the average age of sexarche was 16.75 years old.

The use of IFN to treat CINs started in the 1980s, and perhaps because good clinical results were obtained at that time, most studies examing this therapy date from that decade. One study [16] administered perilesional IFNα and IFNβ to CIN patients and observed that IFNα induced complete remission of the lesion in 85.7% of the patients; of these, 55% recurred 12-24 months after treatment. With IFNβ, complete response occurred in only 40% of the cases. Similar results with the use of IFN were also obtained by DUNHAM *et al*. [17]. Another study [18], which used a methodology similar to ours, obtained complete response in 33% of the cases, partial regression in 58% of cases, and therapeutic failure in 8% of cases. The cure of a patient with invasive vaginal neoplasia using intralesional IFNα-2b has also been reported [19]. However, other studies [22-23] that applied IFNα gel topically and IFNα-2b intralesionally obtained results similar to a placebo.

In our study group (n = 8), we observed that 62.5% of the patients showed good response to treatment, with the high-grade lesion disappearing, while 37.5% of the patients had therapeutic failure, though we did not track the patients over a long period of time. In terms of colposcopic evidence of lesion regression, observed during treatment (fragmentation of the mosaic and gradated attenuation of the dense acetowhite areas of the epithelium with substitution by metaplastic squamous epithelium), our findings are similar to those of Choo *et al*. [16]. Those authors state that they observed changes from the third day of treatment onward, but in our study, we observed these changes later (after the second week of treatment). With respect to lesion size, our findings suggest that smaller lesions have a greater probability of complete response, but the presence of large lesions (those occupying more than one quadrant) does not mean the treatment is contraindicated since 50% of the patients with this type of lesion achieved therapeutic success.

The minor side-effects (fever, headache, myalgia and asthenia) that were observed in all of the patients in this study cohort were consistent with those in the literature [18]. We did not observe any major side-effects (changes in the central nervous system, cardiopathy, myelosuppression). Nor were there any cases of changes in hemogram, coagulogram, or liver and kidney enzymes.

Increases in the production of IL-10 and IL-4 may be a mechanism that tumor cells use to escape recognition by the immune system [24, 25]. Indeed, IL-10 and TGF-β inhibit the maturation of dendrite cells and can indirectly block the response of T cytotoxic lymphocytes [26]. The biological effects of IL-10 stem from its capacity to inhibit the functions of activated macrophages, in addition to inhibiting the expression of class II major histocompatibility molecules in macrophages, which, in turn, reduce the activation of T cells and cell immunity. IL-4, the principal stimulus for the development of Th2 cells, can prejudice IFN-γ's effect on activating macrophages and thereby inhibit cellular immune reactions. In our study, higher initial concentrations of IL-10 were observed in patients with treatment failure. Although these concentrations fell during treatment, they remained elevated compared to the group of patients who responded well to therapy. While it has not been statistically proven, this pattern of observations suggests that higher levels of IL-10 may be related to treatment failure. Low levels of IL-4 were observed in all patients during treatment, with no significant difference in concentration between the responder and non-responder groups.

IL-6, which acts on both innate and acquired responses, was present in relatively high concentrations in patients whose therapy failed (on average > 500 pg /ml) and at lower levels in patients whose therapy was efficacious ($p = 0.357$ Mann Whitney test, after the sixth application). A recent study observed an increase in the concentrations of IL-6 and IL-8 in the vaginal secretion of CIN patients [27]. It has already been shown that the expression of IL-6 in cervical cancer is related to tumor size (> 2 cm) and that this expression promotes tumor angiogenesis and

encourages the development of cervical cancer [28]. In our study, all patients whose IFN therapy failed had a lesion that occupied more than one quadrant, which might explain the increase in the expression of this cytokine in this group.

An increase in the concentration of IL-8 in the vaginal secretion of patients with cervical cancer has already been observed, with this cytokine being considered pro-inflammatory [29]. Some evidence suggests that this IL-8 may play a fundamental role in angiogenesis and may be associated with advanced cervical tumors [30]. Although our study did not note a significant difference in the concentration of IL-8 between the groups (failed vs responsive), it did show higher levels of IL-8 concentration in both groups (on average > 4000 pg/ml), which is consistent with the literature [27].

Some studies have noted an increase in the concentration of TNF-α in patients with CIN [31, 32]. However, other studies [33-35] have observed a systemic decrease of the Th1 cytokines (IFN-γ, TNF-α, and IL-2), which is correlated to an increase in CIN grade. Scott *et al*. [4] suggest that persistent HPV infection leads to a failure to express Th1 cytokines. In our study, we observed low levels (on average < 15 pg) of IFN-γ, TNF-α, IL-2, and IL-12 in both patients whose therapy failed and in those who responded well to treatment. These findings reinforce the idea that a decrease in Th1 cytokines occurs in high-grade lesions, as all of our patients had a high-grade CIN diagnosis.

We observed that IL-1β levels were higher in the group with therapeutic failure than in the group that responded well to treatment, but they were not statistically significant. IL-1β is produced by different cell types, including keratinocytes, and acts on local inflammation. On the basis of the present study, we suggest that very high levels of this IL are a factor in the failure of intralesional IFN therapy. Further studies, involving a larger number of patients, are needed to better clarify the pattern of immune response involved in treatment with IFNα-2b, although thus far it seems likely that the levels of more inflammatory cytokines may correlate with intralesional IFNα-2b treatment failure than with treatment success.

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## References

[1] Parkin D.M., Bray F.: "The burden of HPV-related cancers". *Vaccine,* 2006, *3,* S3/11.

[2] McCredie M.R., Sharples K.J., Paul C., Baranyai J., Medley G., Jones R.W., Skegg D.C.: "Natural hitory of cervical neoplasia and risk of invasive cancer in women with cervical Intraepithelial neoplasia 3: a retrospective cohort study". *Lancet Oncol*., 2008, *9*, 425.

[3] Woodman C.B., Collins S.I., Young L.S.: "The natural history of cervical HPV infection: unresolved issues". *Nat. Rev. Cancer.,* 2007, *7*, 11.

[4] Scott M., Stites D.P., Moscicki, A.B.: "Th1 cytokine patterns in cervical human papillomavirus infection". *Clin. Diagn. Lab. Immunol.,* 1999, *6,* 751.

[5] Frazer I.H., Thomas R., Zhou J., Leggatt G.R., Dunn L., McMillan N. *et al*.: "Potential strategies utilised by papillomavirus to evade host immunity". *Immunol. Rev*.,1999, *168,* 131.

[6] Tindle R.W.: "Immune evasion in human papillomavirus-associated cervical cancer". *Nat. Rev. Cancer,* 2002, *2,* 59.

[7] Wu T.C., Kurman R.J.: "Analysis of cytokine profiles in patients with human papillomavirus-associated neoplasms". *J. Natl. Cancer Inst.,* 1997, *89*, 245.

[8] Gonçalves M.A., Donadi E.: "A immune cellular response to HPV: current concepts". *Braz. J. Infect. Dis.,* 2004, *8,* 1.

[9] Wright T.C. Jr., Massad L.S., Dunton C.J., Spitzer M., Wilkinson E.J., Solomon D.: American Society for Colposcopy and Cervical Pathology-Sponsored Consensus Conference. "2006 Consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ". *Am. J. Obstet. Gynecol*., 2007, *197,* 340.

[10] Jakobsson M., Gissler M., Sainio S., Paavonen J., Tapper A.M.: "Preterm delivery after surgical treatment for cervical intraepithelial neoplasia". *Obstet. Gynecol*., 2007, 309.

[11] Kyrgiou M., Koliopoulos G., Martin-Hirsch P., Arbyn M., Prendiville W., Paraskevaidis E.: "Obstetric outcomes after conservative treatment for intraepithelial or early invasive cervical lesions: systematic review and meta-analysis". *Lancet*, 2006, *367*, 489.

[12] Sadler L., Saftlas A.: "Cervical surgery and preterm birth". *J. Perinat. Med.,* 2007, *35*, 5.

[13] Sjoborg K.D., Vistad I., Myhr S.S., Svenningsen R., Herzog C., Kloster-Jensen A. *et al*.: "Pregnancy outcome after cervical cone excision: a case-control study". *Acta Obstet. Gynecol. Scand.,* 2007, *86,* 423.

[14] Moscicki A.B: "HPV infections in adolescents". *Dis. Markers.,* 2007, *23,* 229.

[15] Nomelini R.S., Mardegan M.C., Murta E.F.C.: "Utilization of interferon in gynecologic and breast cancer." *Clin. Med. Oncol.,* 2007, *1*, 111.

[16] Choo Y.C., Seto W.H., Hsu C., Tany Y.H., Ma H.K., Ng M.H.: "Cervical intraepithelial neoplasia treated by perilesional injection of interferon". *Br. J. Obstet. Gynaecol*., 1986, *93*, 372.

[17] Dunhan A.M., McCartney J.C., McCance D.J., Taylor R.W.: "Effect of perilesional injection of α-interferon on cervical intraepithelial neoplasia and associated human papillomavirus infection. *J. R. Soc. Med*., 1990, *83,* 490.

[18] Stellato G.: "Intralesional recombinant alpha 2B interferon in the treatment of human papillomavirus-associated cervical intraepithelial neoplasia". *Sex. Transm. Dis*., 1992, *19*, 124.

[19] Murta E.F.C., Tavares Murta B.M.: "Successful pregnancy after vaginal cancer treated with interferon". *Tumori*, 2004, *90,* 247.

[20] Castellsaqué X., Muñoz N.: "Cofactors in human papillomavirus carcinogenesis-role of parity, oral contraceptives, and tobacco smoking". *J. Natl. Cancer Inst. Monogr*., 2003, *31,* 20.

[21] Ault K.A.: "Epidemiology and natural history of human papillomavirus infections in the female genital tract". *Infect. Dis. Obstet. Gynecol*., 2006, suppl. *40470*, 1.

[22] Byrne M.A., Moller B.R., Taylor-Robinson D., Harris J.R.W., Wickenden C., Ickenden C. *et al*.: "The effect of interferon on human papilloma virus associated with cervical intraepithelial neoplasia". *Br. J. Obstet. Gynaecol*., 1986, *93,* 1136.

[23] Frost L., Skajaa K., Hvidman L.E., Fay S.J., Larsen P.M.: "No effect of intralesional injection of interferon on moderate cervical intraepithelial neoplasia". *Br. J. Obstet. Gynecol*., 1990, *97,* 626.

[24] Clerici M., Merola M., Ferrario E., Trabattoni D., Villa M.L., Stefanon B. *et al*.: "Cytokine production patterns in cervical intraepithelial neoplasia: association with human papillomavirus infection". *J. Natl. Cancer Inst.,* 1997, *89,* 245.

[25] Seo N., Hayakawa S., Takigawa M., Tokura Y.: "Interleukin-10 expressed at early tumour sites induces subsequent generation of CD4(+) T-regulatory cells and systemic collapse of antitumour immunity". *Immunology*., 2001, *103,* 449.

[26] Loskog A., Ninalga C., Totterman T.H.: "Dendritic cells engineered to express CD40L continuously produce IL 12 and resist negative signals from Tr1/Th3 dominated tumors". *Cancer Immunol. Immunother*., 2006, *55,* 588.

[27] Tavares-Murta B.M., De Resende A.D., Cunha F.Q., Murta E.F.C.: "Local profile of cytokines and nitric oxide in patients with bacterial vaginosis and cervical intraepithelial neoplasia". *Eur. J. Obstet. Gynecol. Reprod. Biol*., 2008, *138*, 93.

[28] Wei L.H., Kuo M.L., Chen C.A., Cheng W.F., Cheng S.P., Hsieh F.J., Hsieh C.Y.: "Interleukin-6 in cervical cancer: the relationship with vascular endothelial growth factor". *Gynecol. Oncol.,* 2001, *82,* 49.

[29] Tjiong M.Y., Van Der Vange N., Ten Kate F.J., Tjong-A-Hung S.P., Ter Schegget J., Burger.M.P., Out T.A.: "Increased IL-6 and IL-8 levels in cervicovaginal secretions of patients with cervical cancer". *Gynecol. Oncol*., 1999, *73,* 285.

[30] Fujimoto J., Sakaguchi H., Aoki I., Tamaya T.: "Clinical implications of expression of interleukin 8 related to angiogenesis in uterine cervical cancers". *Cancer Res*., 2000, *60,* 2632.

[31] Azar K.K., Tani M., Yasuda H., Sakai A., Inoue M., Sasagawa T.: "Increased secretion patterns of interleukin-10 and tumor necrosis factor-alpha in cervical squamous intraepithelial lesions". *Hum. Pathol*., 2004, *35*,1376.

[32] Pardo-Govea T., Callejas D., Núñez-Troconis J., Araujo M., Costa L., Pons H. *et al*.: "Gamma interferon (IFN-gamma), tumor necrosis factor alpha (TNF-alpha) and interleukins 2, 4 and 6 (IL-2, IL-4, IL-6) in cervical-uterine cells of intraepithelial neoplasia: a preliminary report". *Invest. Clin.,* 2005, *46,* 5.

[33] Bais A.G., Beckmann I., Ewing P.C., Eijkemans M.J., Meijer C.J., Snijders P.J. *et al*.: "Cytokine release in HR-HPV(+) women without and with cervical dysplasia (CIN II and III) or carcinoma, comparared with HR- HPV(-) controls". *Mediators Inflamm.,* 2007, *2007,* 1.

[34] El-Sherif A.M., Seth R., Tighe P.J., Jenkins D.: "Quantitative analysis of IL-10 and IFN-gamma mRNA levels in normal cervix and human papillomavirus type 16 associated cervical precancer". *J. Pathol*., 2001, *195,* 179.

[35] Lee B.N., Follen M., Shen D.Y., Malpica A., Adler-Storthz K., Shearer W.T., Reuben J.M.: "Depressed type 1 cytokine synthesis by superantigen-activated CD4+ T cells of women with human papillomavirus-related high-grade squamous intraepithelial lesions". *Clin. Diagn. Lab. Immunol*., 2004, *11,* 239.

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