

Efficacy of carboximethyl beta-glucan in the regression of HVP correlated, low-grade cervical cytological alterations

P. SCARDAMAGLIA ^{1,2}, C. CARRARO ^{1,2}, P. MANCINO ^{1,2}, P. STENTELLA ^{1,2}

EFFECTIVENESS OF THE TREATMENT WITH BETA-GLUCAN IN THE HPV-CIN1 LESIONS

Aim. The aim of the study was to evaluate the effectiveness of the beta-glucan in women with abnormal cytology, including the women with a positive screening for ASCUS-LSIL furtherly divided in women with positive cytology (ASCUS or LSIL) and negative colposcopy and women with abnormal cytology, positive colposcopy and human papilloma virus (HPV)-CIN1 histology who opted for follow-up.

Methods. From September 2007 to December 2008, 60 women with ASCUS-LSIL diagnosis were recruited at the ambulatory of Lasersurgery and Cervico-Vaginal Patology, Department of Gynecology and Obstetrics of Policlinico Umberto I of Rome. The women was subdivided in two groups: 1) women with cytological diagnosis of ASCUS or LSIL and negative colposcopy; 2) women with abnormal cytology, positive colposcopy and HPV-CIN1 histology, who opted for follow-up. All the women were treated with two cycles of a daily topical application of beta-glucan for 20 consecutive days with a suspension of 10 days. The effects of beta-glucan were analyzed with colposcopy and cytology at 3, 6 and 12 months from the beginning of the therapy.

Result. After 3 months of treatment, of the 30 women with positive cytology and negative colposcopy, 80% with ASCUS diagnosis resulted negative, 35% with LSIL diagnosis resulted

¹Ambulatory of Lasersurgery and Cervico-Vaginal Patology,

Sapienza University, Rome, Italy

²Department of Gynecological Studies, Perinatology and Neonatal Nursing,

Umberto I Polyclinic, Rome, Italy.

negative; after 6 months 100% with ASCUS diagnosis resulted negative, 70% with LSIL diagnosis resulted negative; after 12 months 85% with LSIL diagnosis resulted negative. Of the 30 women with positive cytology, positive colposcopy and HPV-CIN1 histology after 3 months 20% resulted negative, after 6 months 60% resulted negative and after 12 months 80% resulted negative. The persistence of the HPV-CIN1 histology was verified in the 13% of the women. For these women the definitive treatment was the TFD.

Conclusion. Our study demonstrate the effectiveness of the treatment with beta-glucan in the women with ASCUS-LSIL lesions and HPV-CIN1 lesions, increasing of the regressions rate after 12 months of the treatment of the 15-20%.

Key words: Beta-glucans - Colposcopy - Cell biology.

The correlation between genital infection with human papillomavirus (HPV) and cervical cancer was first theorized in the early 1980s by German virologist Harald zur Hausen and later confirmed by numerous authors throughout the following years.

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Contact author: P. Scardamaglia, Policlinico Umberto I, Viale del Policlinico, 00100, Rome, Italy.
E-mail: paola.scr@libero.it

Cervical cancer was the first type of cancer to be recognized by the World Health Organization (WHO) as entirely attributable to an infection^{1,3}.

As of today, more than 100 HPV genotypes that infect humans have been identified. Of these, about one third are associated with diseases of the genital tract, both benign and malignant. HPV types are referred to as low-risk (human papillomavirus low risk, HPV-LR) and high-risk (human papillomavirus high risk, HPV-HR) of neoplastic transformation. This distinction is made due to the fact that the transforming capacity of the virus is limited to the persistent infection of the high-risk subtypes which can integrate their genome into the one of the host cell and interact with the delicate mechanisms of cellular replication⁴.

The high-risk viral genotypes most frequently connected to cervical cancer are type 16 (50%) and type 18 (20%), accounting for up to 70% of cases, the remaining types (31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73, 82...) account for the remaining 30% of neoplasms^{5,6}.

HPV infection mostly affects women. More than 75% of sexually active women become infected in their lifetime, with a peak shortly before the age of 25. The natural history of the viral infection is correlated to the functioning of the patients' immune system. In most cases it is a temporary infection, given that the virus is cleared naturally by well-functioning immune systems within 12 to 24 months. In the remainder of cases viral persistence develops into dysplastic lesions and even cervical cancer, through a gradual process which, to completely unfold, may take several years^{8,9}.

This is why the incidence of cervical cancer increases from the fourth decade of life and is not frequent among younger women, among whom, by contrast, HPV infection has a high prevalence. Moreover, there are a number of co-factors affecting the natural history of the viral infection, leading to an increase in the risk of developing dysplastic lesions among HPV positive women. The most studied include: the simultaneous infection with other

sexually transmitted agents (Chlamydia t, Neisseria g, HSV), smoking cigarettes, extensive use of oral contraceptives (more than 5 years), high number of pregnancies (more than 7) and hypovitaminosis^{2,3,6}.

From the literature it has been shown that treating CIN1 does not modify evolutionary distance. Such lesions, caused both by HPV-HR and HPV-LR, regress spontaneously in most cases in few years, above all among young wo-men. Caseof spontaneous regressions without treatment amount to about 50%. Hence, the rationale of waiting with patients under the age of 35 and resorting to a conservative treatment with patients over the age of 35, especially with persistent lesions, large colposcopic lesions and, in any case, of low socio-economic compliance to the follow-up^{8,10}.

While today there are effective treatment methods for evident lesions caused by HPV virus, there is no therapy which can affect the HIV positive status of patients or facilitate a better activity of the immune system of patients in order to improve their response.

Various studies in the literature have proved the existence of a natural substance called beta-glucan, component of the cell wall of numerous yeasts, fungi and bacteria, which is not synthesized by the human body and is therefore recognized as non-self by the immune system. This compound can modulate the tissue immune response through its specific binding to Toll-Like Receptors (TLR), located on the surface of macrophages and dendritic cells. The role of TLRs is to recognize and bind pathogenic antigens¹⁵. TLR activation leads to the phagocytosis of the pathogen and the activation of the metabolism of arachidonic acid with the production of proinflammatory cytokines. These cytokines activate natural-killer (NK) cells and T lymphocytes, thus enhancing the inflammatory response and triggering the specific immune response¹⁸. The binding of beta-glucan to the TLR can modulate the inflammatory response, thereby causing a change in concentration and pattern of cytokines

released with a reduced activation of NK cells and T lymphocytes. This stimulation, which is controlled by the immune activity, protects pathogenic organisms, toxins and environmental carcinogens^{16,17}.

On this basis, the present study aims at evaluating the efficacy of carboxymethyl beta-glucan (Colpofix[®]) on patients with abnormal cytology including patients with cytological examination indicating ASCUS-LSIL, then divided into patients with cytological diagnosis of ASCUS or LSIL and negative colposcopy and patients with abnormal cytology, positive colposcopy and histological examination indicating HPV-CIN1, who, given the choice between physical destructive treatment and follow-up, opted for follow-up.

Materials and methods

At the ambulatory of Lasersurgery and Cervico-Vaginal Pathology, Department of Gynecology, Perinatology and Neonatal Nursing of Sapienza University in Rome, Italy, at the Polyclinic Umberto I, from September 2007 to December 2008, a total of 60 patients with cytological screenings of ASCUS-LSIL were recruited and divided into two subgroups:

1. Patients with cytological diagnosis of ASCUS or LSIL and negative colposcopy;
2. Patients with abnormal cytology, positive colposcopy and histological examination indicating HPV-CIN1, who, given the choice between physical destructive treatment and follow-up, opted for follow-up.

The following points were considered: age, age of coitarche, number of sex partners, frequency of monthly sexual intercourse, the number of deliveries, use of oral contraceptives, smoking. The exclusion criteria concern patients with unsatisfactory colposcopy (SCJ not visible, endocervical or not completely visible lesion), patients who underwent a physical destructive treatment within the

18 preceding months, patients treated for high-grade lesions, immuno-suppressed patients or under cortisone-based or immunosuppressive therapy and pregnant patients. The treatment involves the administration of carboxymethyl beta-glucan sprayable gel with one application per day (5 doses) for 20 days, followed by a 10-day suspension and a further 20-day cycle. It is applied in the vagina through specific disposable applicators, it is applied evenly over the entire vaginal surface, without leaving any residues or causing burning or itching as side effects. The patients were evaluated with a colpocytological examination 3, 6 and 12 months after the end of the therapy. The cytological examination was performed by collecting the material both from the exocervical area, with an Ayre spatula, and from the endocervical area, with a cytobrush; the results were interpreted in accordance with the Bethesda 2001 classification. The colposcopic examination was carried out with a Zeiss colposcope and the colposcopic pictures were interpreted using the international colposcopic classification (Barcelona 2002).

Findings

The average age of the examined patients was 24 (aged 18-35), the average age of coitarche was 17 (age range spanning from 14 to 24), the average number of sex partners 10 (3-20 range), the average of sexual intercourses per week 2 (1-6 range), the average number of deliveries 1 (0-3 range). Moreover, 20% of patients regularly used Estrogen-Progestin drugs and about 40% were smokers. All patients underwent therapy with Colpofix[®] following the suggested dosing regimen. No complications or side effects were reported and no patient discontinued the treatment.

Within the group of patients with positive cytology and negative colposcopy, out of a total of 30, 10 (33%) were diagnosed with ASCUS, 20 (67%) with LSIL. At the first colpocytological screening after 3 months 8 patients out of 10 (80%) with initial ASCUS diagnosis resulted negative, 7 out of 20

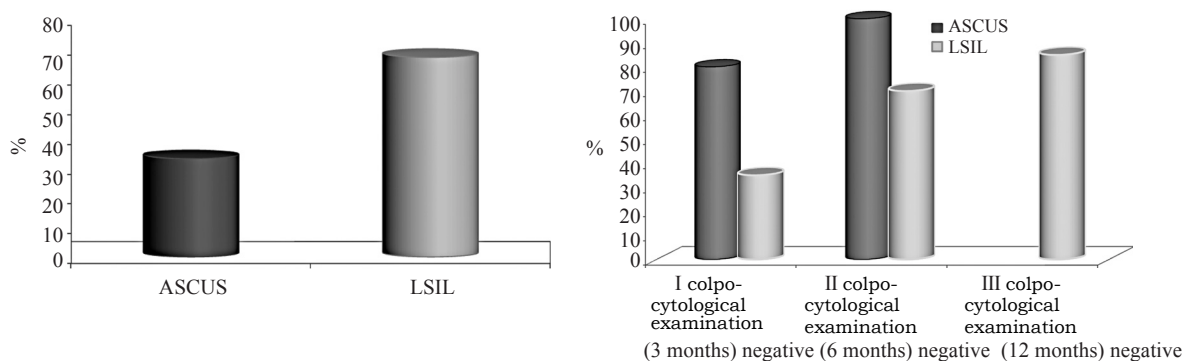


Figure 1. Group with positive cytology and negative colposcopy. A) ASCUS and LSIL B) Colposcopic examinations

patients (35%) with initial LSIL diagnosis resulted negative; at the colposcopic screening after 6 months 10 out of 10 patients (100%) with initial ASCUS diagnosis resulted negative, 14 out of 20 patients (70%) with initial LSIL diagnosis resulted negative; at the screening after 12 months 17 out of 20 patients (85%) with an initial LSIL diagnosis resulted negative while 3 patients (15%) became lost to follow-up (Figure 1).

Of the 30 patients who at the screening presented abnormal cytology, positive colposcopy and histological examination indicating HPV-CIN1 at their first colposcopic screening after 3 months 6 out of 30 patients (20%) resulted negative; at the colposcopic screening after 6 months 18 out of 30 patients (60%) resulted negative; at the colposcopic screening after 12 months 24 out of 30

resulted negative. The patients (80%) persistence of CIN1-HPV, confirmed histologically, was detected in 4 out of 30 patients (13%), who underwent physical destructive therapy as final treatment, while 2 out of 30 patients (6%) became lost to follow-up (Figures 2, 3).

Conclusions

Although, as can be seen from data offered by the international literature, about 5-17% of patients with ASCUS cytological diagnosis and about 15-30% of patients with L-SIL cytological diagnosis may show a final histological diagnosis of CIN II- CIN III, such cases must not be managed aggressively¹⁴. The current guidelines suggest a colposcopic examination after 3 months and a more careful evaluation of the cervical canal and of the vagina in the event of negative colposcopic examination. The percentage of spontaneous of

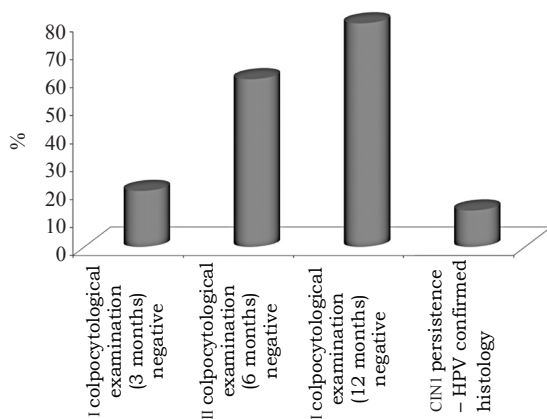


Figure 2. Patients with abnormal cytology, positive colposcopy and HPV-CIN1 histological examination

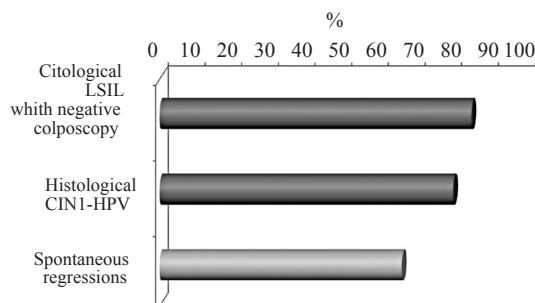


Figure 3. Percentage of regression with carboxymethyl beta-glucan treatment (12 months).

cytological lesions after 3 months amounts to about 40% among young women 14. As regards CIN1-HPV histological diagnosis, the review of the literature indicates two approaches:

— wait and see (colpocytological examinations every 6 months for a maximum of 2 years);

— immediate physical destructive treatment.

The first approach is justified by the high percentage of LSIL spontaneous regressions and by the possibility of intervening successfully in progression cases. The disadvantages of this approach are the state of anxiety the patient experiences throughout the follow-ups, the cases of unchecked progressions and to the need to resort to level 2 specialized centers. By contrast, the advantages of the physical destructive treatment are the immediate treatment of lesions of patients with little compliance to follow-ups and for patients who experience severe anxiety due to the diagnosis. The disadvantages can be correlated to the side effects of physical destructive therapy, costs and possible recurrences^{11, 12}.

As the literature has already clearly demonstrated, the role played by the immune system in determining the regression/progression of lesions, the use of a molecule capable of triggering in a modulated and controlled way the physiological immune response of the human body to pathogens may constitute a valid aid¹⁰. This is what beta-glucan aims to do. Proinflammatory cytokine production triggered by its action and the resulting NK and T lymphocytes activation suggests an enhanced stimulation of the cervical tissue to regeneration and reparation¹⁹⁻²¹. The present study has highlighted, with a six-monthly follow-up of 12 months, among women who regularly used carboxymethyl beta-glucan following the recommended regimen, a complete regression of LSIL cytological lesions, with negative colposcopy, equal to 85%, and the total regression of CIN1-HPV histological lesions equal to 80%. These values, if compared with the percentages of spontaneous regression of the above-mentioned lesions observed in the literature (66-70%), show a significant difference in percentage equal to 15-20%. Medical treatment with carboxymethyl beta-glucan, according to the

therapeutic regimen, for 2 months with colpo-cytological follow-up after 3, 6 and 12 months, would therefore constitute a valid alternative to the immediate physical destructive treatment and to the simple "wait and see" solution, given that it entails good tolerability to the drug, low costs, high compliance by the patients and a clear improvement of the regression index of the lesions after 12 months.

Summary

Objective. The present study aims at evaluating the efficacy of carboxymethyl beta-glucan (Colpofix[®]) on patients with abnormal cytology, including patients with cytology indicating ASCUS-LSIL at the screening, subsequently divided in patients with ASCUS or LSIL cytological diagnosis and negative colposcopy and patients with abnormal cytology, positive colposcopy and histological examination indicating HPV-CIN1, who, after being given the choice between physical destructive treatment and follow-up, opted for follow-up.

Methods. At the ambulatory of Laser-surgery and Cervico-Vaginal Pathology, Department of Gynecology, Perinatology and Neonatal Nursing of Sapienza University in Rome, Italy, at the Polyclinic Umberto I, from September 2007 to December 2008, 60 patients with ASCUS-LSIL diagnosis were recruited and divided into two subgroups: patients with cytological diagnosis of ASCUS or LSIL and negative colposcopy, and patients with abnormal cytology, positive colposcopy and histological examination indicating HPV-CIN1, who, given the choice between physical destructive treatment and follow-up, opted for follow-up. The treatment involves the administration of carboxymethyl beta-glucan sprayable gel with one application per day (5 doses) for 20 days, followed by a 10-day suspension and a further 20-day cycle. The patients were evaluated with a colpocytological examination 3, 6 and 12 months after the end of therapy.

Findings. In the group of patients with positive cytology and negative colposcopy, out of a total of 30, 10 patients (33%) were diagnosed with ASCUS, 20 (67%) with LSIL.

At the first colposcycological examination after 3 months 8 out of 10 patients (80%) with initial ASCUS diagnosis resulted negative, 7 out of 20 patients (35%) with initial LSIL diagnosis resulted negative; at the colposcycological examination after 6 months 10 out of 10 patients (100%) with initial ASCUS diagnosis resulted negative, 14 out of 20 patients (70%) with initial LSIL diagnosis resulted negative; at the examination after 12 months 17 out of 20 patients (85%) with initial LSIL diagnosis resulted negative while 3 patients (15%) became lost to follow-up. Of the 30 patients who at the screening presented abnormal cytology, positive colposcopy and histological examination indicating HPV CIN1 at the first colposcycological examination after 3 months 6 out of 30 patients (20%) resulted negative, at the colposcycological examination after 6 months 18 out of 30 patients (60%) resulted negative, at the colposcycological examination after 12 months 24 out of 30 patients (80%) resulted negative. The persistence of CIN1-HPV, confirmed histologically, was found in 4 out of 30 patients (13%), for whom a physical destructive therapy was chosen as final treatment, while 2 out of 30 patients (6%) became lost to follow-up.

Conclusions. The present study has highlighted, with a six monthly follow-up in 12 months, in women who regularly used carboxymethyl beta-glucan following the recommended regimen, a complete regression of LSIL cytological lesions, with negative colposcopy, equal to 85%, the total regression of CIN1-HPV histological lesions equal to 80%, thus constituting a valid alternative to immediate physical destructive treatment and to the simple "wait and see" approach, due to its good tolerability to the drug, low costs, high compliance by patients and clear improvement of the regression index of the lesions after 12 months.

Key words: Beta-glucans – Colposcopy – Cell Biology

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