# Spontaneous regression of low-grade cervical intraepithelial lesions is positively improved by topical bovine colostrum preparations (GINEDIE®). A multicentre, observational, italian pilot study

C. STEFANI, C.A. LIVERANI[1], V. BIANCO[2], C. PENNA[3],
T. GUARNIERI[4], C. COMPARETTO[5], E. MONTI[1], I. VALENTE[2],
A.L. PIERALLI[3], C. FIASCHI[5], M. ORIGONI

Department of Obstetrics and Gynecology, Vita Salute San Raffaele University School of Medicine, Milan, Italy
[1]Department of Obstetrics and Gynecology, L. Mangiagalli Institute, University of Milan, Italy
[2]Department of Obstetrics and Gynecology, V. Buzzi Hospital, University of Milan, Italy
[3]Department of Obstetrics and Gynecology, Careggi Hospital, University of Florence, Italy
[4]Obstetrics and Gynecology, Istituti Clinici Zucchi, Monza, Italy
[5]Obstetrics and Gynecology, ASL 4 Prato, Italy

**Abstract.** – **OBJECTIVES:** Human papillomavirus (HPV) is the causal agent of cervical cancer. The great majority of abnormal Pap test results – almost 90% – is referrable to either atypical squamous intraepithelial lesion or CIN1. For these lesions, worldwide agreement exists concerning the high rate – ranging from 40% to 70% – of spontaneous regression over a period of 1-5 years. Host's immune response is a key point influencing the natural history of these conditions. Bovine colostrum is a natural agent positively promoting several immune activities against bacterial and viral agents. The aim of this report was to evaluate the potential positive effect of bovine colostrum-containing vaginal tablets administered to CIN1 diagnosed patients in a prospective trial in regards to spontaneous regression rate.

**PATIENTS AND METHODS:** A series of 256 consecutive patients with histologically proven CIN1 recruited in a multicentre, observational, Italian study. Patients have been enrolled in a 24-weeks protocol of treatment and re-tested at the end of the study. Rates of regression have been recorded.

**RESULTS:** Overall regression rate to a negative histology at the end of the 6 month follow up was 75.5%.

**CONCLUSIONS:** Regression to normal histology was observed in a very high rate of cases in a very short period compared to the natural history of these lesions. CIN1 patients could benefit from bovine colostrum topical administration in terms of significantly shortening the regression time.

*Key Words:*
  Human Papillomavirus, HPV, Low-grade CIN, CIN1, Regression, Natural history, Bovine colostrum, Immunomodulation.

## Introduction

Human papillomavirus (HPV) infection was firstly implicated as an etiologic agent for cervical cancer in 1977[1], and it actually is the most common viral sexually transmitted disease in the United States[2]. It causes approximately 11,000 cases of invasive cancers per year[3] in USA but it's also unequivocally linked with cervical preneoplastic lesions, categorized as squamous intraepithelial neoplasia (CIN) of various degrees. Although the incidence of cervical cancer has dramatically decreased, there has been a steady rise in intraepithelial neoplasia detection worldwide. Approximately 90% of abnormal Pap smears are either atypical squamous intraepithelial lesions or CIN1[4]. Stated the large number of CIN1, many studies about treatment, follow-up and costs of CIN1 management have been performed. A large amount of data agree with a high rate of spontaneous regression[2,4-6] showing that the spontaneous resolution of CIN1 is the rule rather than the exception. Studies on the natural history of these abnormalities show that regression to normal is around 50% for CIN1[7]. More, a comprehensive literature review highlighted that spontaneous regression occurs in 57% of patients[8]. Thus, great interest is focused on the timing of CIN1 regression. A cohort study of more than 17,000 women found that spontaneous regression occurred in 44% of patients within 2 years and in 74% after 5 years of follow-up. These data were confirmed in a large group of patients, showing a

52% of CIN1 regression within 1 year of follow-up[10]. Management of CIN1 lesions follow-up has been largely discussed in multiple studies. Despite low agreement exists on which test better fits for CIN1 follow-up, all recent studies agree with the difficulty in achieving patient's compliance for such a long follow-up[11]. Due to this aspect, together with the long-term follow-up costs, great interest is given in obtaining shorter regression time of these lesions; since the host immune response is recognized as a key point influencing natural history of HPV-related cervical intraepithelial lesion, many efforts have been dedicated to immunostimulating agents. Colostrum is the pre-milk fluid produced by female mammary glands immediately after delivery. The bovine and human colostrum contains several immunological factors that confer the first immunization of the newborn, protecting against many microbial pathogens[12]. Colostrum is a highly complex mixture of various effector molecules; this explains the wide range of pharmacodynamic effects described in *in-vitro* systems, both in animals and in clinical studies; main actions include an antibacterial effect and a positive modulation of the immune response[13]. Bovine colostrum provides almost ninety useful components. The most interesting bioactive substances are immune factors ($\gamma$-globulins or immunoglobulins or antibodies)[14,15] and growth factors (EGF, TGF, FGF, IGF-I, IGF-II, TGF-$\alpha$ and TGF-$\beta$), but also antimicrobial (lactoferrin), cytokines, immunomodulatory substances (colostrinin or proline-rich-polypeptide) and nutrients (vitamin A, E, B12)[16]. Colostrum has already been tested in many clinical trials and its therapeutic effect has been largely demonstrated in gastrointestinal disorders, enteric infections, respiratory tract infections and various chronic infections as bacterial, viral, parasitic or fungal[17]. Furthermore colostrum promotes a positive immune response towards intracellular pathogens such as bacteria and viruses, enhancing IL-12 and IFN-$\gamma$ production, cytokines involved in the specific Th1 response[18].

In this study we investigated the potential positive immunomodulating action of bovine clostrum through vaginal administration in cases of CIN1 followed up according to guidelines of management of these lesions.

## Patients and Methods

The study has been performed during a 2 year period (2012-2013) with the participation of 6 groups: (1) Department of Obstetrics & Gynecology of San Raffaele Institute, Vita Salute San Raffaele University, Milan; (2) Department of Obstetrics & Gynecology of L. Mangiagalli Institute, University of Milan; (3) Department of Obstetrics & Gynecology of V. Buzzi Hospital, University of Milan; (4) Department of Obstetrics & Gynecology of Careggi Hospital, University of Florence; (5) Obstetrics and Gynecology Unit of Istituti Clinici Zucchi, Monza, and (6) Obstetrics and Gynecology Unit of ASL 4, Prato, Italy. The study design comprised for enrollment of cytologically referred and histologically proven new cases of low-grade cervical intraepithelial lesion (CIN1), that have been topically treated with bovine-colostrum containing vaginal tablets (Ginedie® – Tfarma, Florence – Italy) for a 6 month period, and re-tested. The recruited cases came from screening settings and should not having had a previous episode of cervical intraepithelial neoplasia of any degree in the previous two years prior to the study. At baseline and at study end patients have been tested with cervical cytology (Pap smear), colposcopy and targeted biopsy; in some cases high-risk HPV-DNA testing (hrHPV) was also obtained. In colposcopically negative cases, a random biopsy of the cervical transformation zone (TZ) was obtained. Treatment protocol comprised for administration of vaginal tablets twice/week at bedtime for a total of 24 weeks, without any other medication/detergent topical use for the whole study period. Informed consent was locally obtained for any participant.

### Statistical Analysis

Results have been statistically analyzed in terms of histological regression vs. persistence vs. progression rate of cervical lesions with the Chi-square and Fisher *t*-test, assuming a $\alpha$ value of 0.05 as significant.

## Results

An overall of 256 cases represents the studied population (mean age 37.7 yrs.). All the cases had a cytological cervical smear that indicated: 207 (80.85%) cases of L-SIL, 43 (16.79%) cases of ASC-US, 4 (1.56%) cases of H-SIL and 2 (0.78%) negative cases (Figure 1). As far as it concerned histology at baseline, 92.96% of cases (n. 238) were diagnosed as CIN1, 1.95% (n. 5) were diagnosed as CIN1 with isolated cells suggestive for
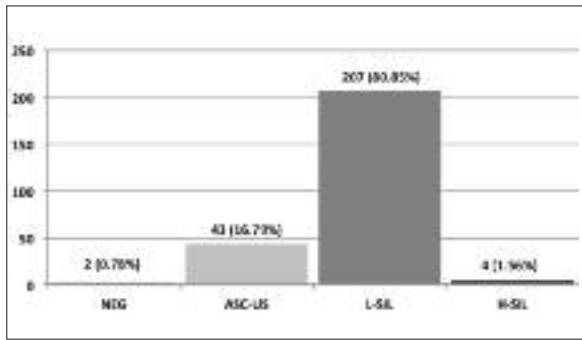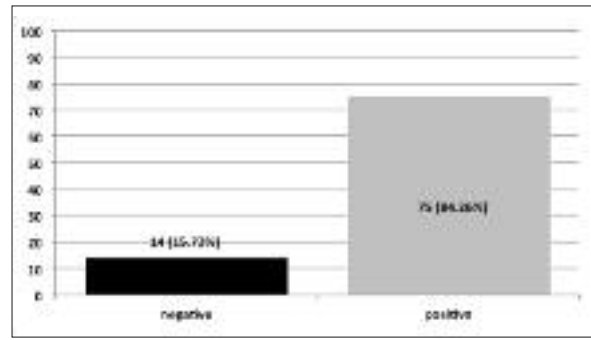
**Figure 1.** Cytology at baseline.



**Figure 3.** hrHPV at baseline (89 cases).

CIN2, and 5.07% (n. 13) were identified as CIN2 (Figure 2). Out of the 256 recruited cases, a subgroup of 89 was also submitted to high-risk HPV-DNA testing both at baseline and at study end; 84.26% of these cases (n. 75) tested positive for hrHPV at baseline (Figure 3). At the end of the treatment protocol, the tests performed were as follows: colposcopic grade 0 of the cervical TZ changed from 19.9% at baseline to 54.2% after treatment, grade 1 changed from 68.7% to 44.1%, and grade 2 changed from 10.9% to 14.0% before and after treatment respectively (Figure 4), with a statistically significative difference ($p < 0.0001$). When final histology was considered, the following results were obtained: CIN1 cases (n. 238) were found to be histologically negative in 71%, persistently CIN1 in 26.8%, and progressed to CIN2 in 2.1% of cases; cases of CIN1/2 (n. 5) at baseline histology were found to be histologically negative in 80% and purely CIN1 in 20% of cases respectively; cases of CIN2 (n. 13) were found histologically negative in 7.6%, regressed to histological CIN1 in 61.5% and persistently CIN2 in 30.7% of cases respectively (Figure 5); statistical analysis of these results demonstrated a strong sig-
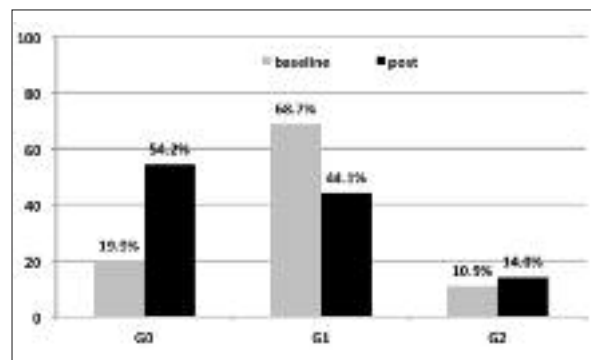


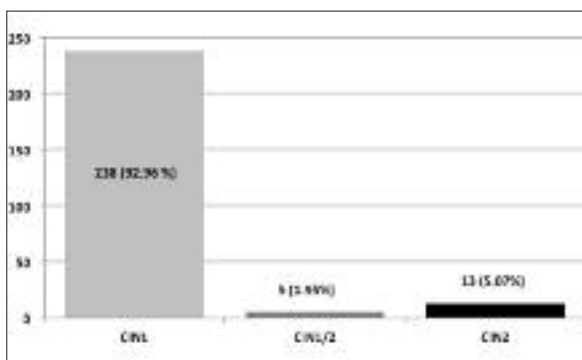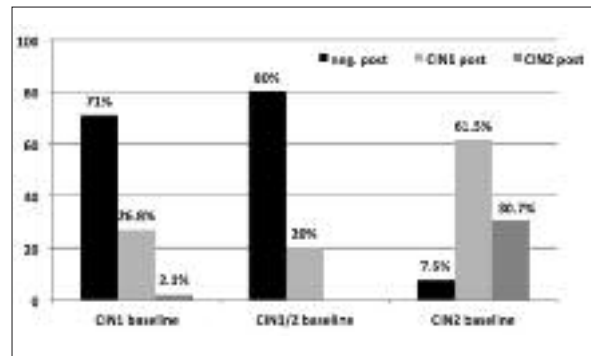**Figure 4.** Colposcopic grade at baseline and post-treatment ($p < 0.0001$).



**Figure 5.** Histology at baseline and post-treatment ($p < 0.0001$).

nificative difference ($p < 0.0001$) comparing baseline to post-treatment. Lastly, as far as it concerned hr-HPV status, CIN1 cases that tested positive for HPV-DNA at baseline (n. 75) were found to be histologically negative in 73.3%, persistently CIN1 in 21.3%, and progressed to histological CIN2 in 5.3% of cases respectively; on the other hand, CIN1 cases that tested negative for HPV-DNA (n. 14) where found to be histologically neg-



**Figure 2.** Histology at baseline.

ative in 78.5% and persistently CIN1 in 21.4%; no cases progressed to histological CIN2 in this subset of patients (Figure 6); in these comparisons, statistical analysis was very close to have reached a significative difference ($p = 0.05$).

## Discussion

In the last decade, very strong evidences have been demonstrated in terms of optimizing diagnostic and management strategies for the effective prevention of invasive cervical cancer. In this view, the key point that emerged from large controlled clinical trials has been the higher performance of HPV DNA testing compared to cervical cytology in detecting preinvasive cervical lesions[19,20]. Also, in consideration of the causal role of HPV for the development of preinvasive and invasive cervical diseases, the most recent guidelines for the management of positive cases have been updated in relation to the natural history of HPV-correlated lesions[21,22]; in particular, a very less active and aggressive management of low-grade lesions has been identified as a rule, due to the high rate of spontaneous regression to normality within 2-5 years[8,9] of follow up. Despite this, wait-and-see management may be correlated with deficient patients' compliance in attending a long-term follow-up protocol with an expected rate of spontaneous regression that, in a woman's mind, may not be seen as particularly reassuring; in fact, guidelines state that ablative treatment of a low-grade intraepithelial lesion (CIN1) is an acceptable option in cases of long-lasting – 2 years – persistence[22]. Many factors are implicated in the process of clearance vs. persistence vs. progression of a cervical intraepithelial preneoplastic lesion: of these, virus-correlated factors



**Figure 6.** hrHPV status and histology post-treatment ($p = 0.05$).

and host-correlated factors are the most important and widely investigated. HPV viral load, for example, has been demonstrated to directly correlate with the severity of the lesion and, supported by recently published experiences, can easily be obtained with simple, cost-effective and validated biomolecular HPV-DNA testing assays, without the need to perform hardly affordable laboratory tests[23-25]; on the other side, the immunological profile of cervical tissues has similarily been studied and very recent results established particular patterns of mucosal immunity having significant prognostic significance in terms of complete regression vs. recurrence of high-grade (CIN2-3) lesions after conservative treatment[26-28]. In this context, drug-induced immunosuppression (e.g. transplanted recipients) has recently been reported to have less importance[29]. Bovine colostrum has been demonstrated, either in animal models and in clinical studies in humans, to own a wide range of positive immunomodulating effects against bacterial and viral agents[12-18]; for this reasons we investigated the potential usefulness of a vaginal preparation (Ginedie® vaginal tablets – Tfarma, Florence – Italy) containing bovine colostrum in cases of histologically proven CIN1. This study represents the first experience in which this preparation has been tested in such cases. In our study, the overall regression rate of lesions to a negative histology in a 6-month period has been 75.5% ($p < 0.0001$). This result, per se, is consistent with what is generally published in literature, where a regression rate of 44-74% has been reported in follow up ranging from 1 to 5 years[7-10]. Supporting this result, the colposcopic grade of the lesions at baseline vs. post-treatment evidenced a 34% increase of grade 0 and a 24% decrease of grade 1 ($p < 0.0001$). Of particular interest, 80% of complete regression to negative histology was obtained in the group of patients with CIN1/2 ($p < 0.0001$). As far as it concerned the hrHPV-DNA positivity of cases at baseline, with the limitation of the small number of patients investigated (89 cases), we did not observed a statistically significant difference in terms of regression to negative histology; this is probably to be correlated with the small sample, as the statistical analysis resulted very close to reach a statistical significance ($p = 0.05$). It is noteworthy to underline two important aspects of our results: firstly, the overall regression rate of our cases can be highlighted as one of the highest ever reported in previous experiences; secondly and most relevant,
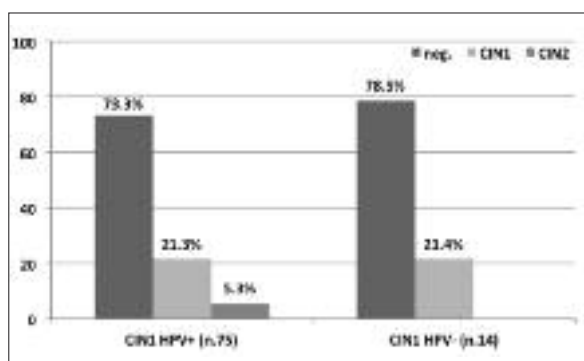
regression rates around 70% have only been reported in very long follow up periods (5 years)[9], while in our study has been obtained in 6 months. This aspect seems to be particularly interesting in consideration of the known difficulties in obtaining patients' compliance and acceptance of long lasting observation follow up periods[11]. Moreover, the issue of follow up-related costs can be interestingly and positively influenced. With the clear study limitation of being observational, it is reasonable to hypothesize that the immunomodulating positive effects of bovine colostrum may have increased and improved, at least in terms of time if not of effectiveness, the rate of regression to normality of our cases. This may particularly be correlated with the promotion of a positive immune response towards intracellular pathogens such as bacteria and viruses, enhancing IL-12 and IFN-γ production, cytokines involved in the specific Th1 response[18]; in fact, a positive effect of high levels of IFN-γ production has previously been demonstrated to correlate with favourable outcomes after treatment of high-grade CIN[28].

## Conclusions

Bovine colostrum represents a very interesting natural agent with potentially highly effective implications in clinical practice[12-14,17] in a wide range of situations, including the approach to cervical intraepithelial neoplasia (CIN), as our findings suggest. If further results from controlled trials will confirm this first pilot experience, a promising, interesting, safe and cost-effective novel approach to the conservative management of low-grade cervical intraepithelial lesions (CIN1) could be proposed in clinical practice.

_____

### Conflict of Interest

The Authors declare that there are no conflicts of interest.

## References

1) Meisels A, Fortin R, Roy M. Condylomatous lesions of the cervix. II. Colposcopic and histopathologic study. Acta Cytol 1977; 21: 379-390.

2) Hoskins WJ, Perez CA, Young RC (Eds.). Principles and practice of gynecologic oncology. JB Lippincott Ed., Philadelphia, 1992.

3) http://www.cancer.org

4) Falls RK. Spontaneous resolution of grade 1 cervical intraepithelial neoplasia in a private practice population. Am J Obstet Gynecol 1999; 181: 278-282.

5) Hamm RM, Loemker V, Reilly KL, Johnson G, Dubois P, Staveley-O'Carroll K. A clinical decision analysis of cryotherapy compared with expectant management for cervical dysplasia. J Fam Pract 1998; 47: 193-201.

6) Baldauf JJ, Ritter J. Comparison of the risks of cytologic surveillance of women with atypical cells or low-grade abnormalities on cervical smear: review of the literature. Eur J Obstet Gynecol Reprod Biol 1998; 76: 193-199.

7) Melnikow J, Nuovo J, Willan AR. Natural history of cervical squamous intraepithelial lesions: a meta-analysis. Obstet Gynecol 1998; 92: 727-745.

8) Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol 1993; 12: 186-192.

9) Holowaty P, Miller AB, Rohan R and To T. Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst 1999; 91: 252-257.

10) Bansal N, Wright JD, Cohen CJ, Herzog TJ. Natural history of established low grade cervical intraepithelial (CIN 1) lesions. Anticancer Res 2008; 28(3B): 1763-1766.

11) ASCUS-LSIL Triage Study (ALTS) Group. A randomized trial on the management of low-grade squamous intraepithelial lesion cytology interpretations. Am J Obstet Gynecol 2003;188: 1393-1400.

12) Henderson DR, Mitchell D. Colostrum: nature's healing miracle. CNR Publications, Salt Lake City, UT, USA 1999.

13) Struff WG, Sprotte G. Bovine colostrums as a biologic in clinical medicine: a review. Part II: clinical studies. Int J Clin Pharmacol Ther 2008; 46: 211-225.

14) Kelly GS. Bovine colostrums: a review of clinical uses. Altern Med Rev 2003; 8: 378-394.

15) Korhonen H, Marnila P, Gill HS. Milk immunoglobulins and complement factors. Br J Nutr 2000; 84(Suppl 1): S75-80.

16) Playford RJ, Macdonald CE, Johnson WS. Colostrum and milk-derived peptide growth factors for the treatment of gastrointestinal disorders. Am J Clin Nutr 2000; 72: 5-14.

17) Thapa BR. Therapeutic potentials of bovine colostrums. Ind J Ped 2005; 72: 849-852.

18) Biswas P, Vecchi A, Mantegani P, Mantelli B, Fortis C, Lazzarin A. immunomodulatory effects of bovine colostrum in human peripheral blood mononuclear cells. New Microbiol 2007; 30: 447-454.

19) Origoni M, Cristoforoni P, Costa S, Mariani L, Scirpa P, Lorincz A, Sideri M. HPV-DNA testing for cervical cancer precursors: from evidence to clinical practice Ecancermedicalscience 2012; 6: 258 doi: 10.3332/ecancer.2012.258

20) MARIANI L, SIDERI M, COSTA S, CRISTOFORONI P, ORIGONI M, PRETI M. Human papillomavirus DNA and pap tests: The need for cotesting in opportunistic setting during the transition time J Low Gen Tract Dis 2013; 17: 362-365.

21) SASLOW D, SOLOMON D, MD, LAWSON HW, KILLACKEY M, KULASINGAM SL, CAIN J, GARCIA FAR, MORIARTY AT, WAXMAN AG, WILBUR DC, WENTZENSEN N, DOWNS LS JR, SPITZER M, MOSCICKI AB, FRANCO EL, STOLER MH, SCHIFFMAN M, CASTLE PE, MYERS ER. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology Screening Guidelines for the Prevention and Early Detection of Cervical Cancer. Am J Clin Pathol 2012; 137: 516-542.

22) MASSAD LS, EINSTEIN MH, HUH WK, KATKI HA, KINNEY WK, SCHIFFMAN M, SOLOMON D, WENTZENSEN N, AND LAWSON HW, FOR THE 2012 ASCCP CONSENSUS GUIDELINES CONFERENCE. 2012 Updated Consensus Guidelines for the Management of Abnormal Cervical Cancer Screening Tests and Cancer Precursors. J Low Gen Tract Dis 2013; 17: S1-S27.

23) ORIGONI M, CARMINATI G, ROLLA S, CLEMENTI M, SIDERI M, SANDRI MT, CANDIANI M. Human papillomavirus viral load expressed as relative light units (RLU) correlates with the presence and grade of preneoplastic lesions of the uterine cervix in atypical squamous cells of undetermined significance (ASCUS) cytology. Eur J Clin Microbiol Infect Dis 2012; 31: 2401-2406.

24) ORIGONI M, CARMINATI G, SIDERI M, CLEMENTI M, ROLLA S, CANDIANI M. "Low-grade positivity" of HPV viral load after atypical squamous cells of undetermined significance (ASC-US) cytology identifies women at low-risk for cervical intraepithelial neoplasia grade 2 and 3. Eur J Gynaecol Oncol 2012; 33: 261-264.

25) ORIGONI M. High viral load identifies cervical lesions. Cancer Epidemiol Biom Prev 2013; 22: 1924.

26) SERESINI S, ORIGONI M, CAPUTO L, LILLO F, LONGHI R, VANTINI S, PAGANONI AM, PROTTI MP. CD4+ T cells against human papillomavirus-18 E7 in patients with high-grade cervical lesions associate with the absence of the virus in the cervix. Immunology 2010; 131: 89-98.

27) ORIGONI M, PARMA M, DELL'ANTONIO G, GELARDI C, STEFANI C, SALVATORE S, CANDIANI M. prognostic significance of immunohistochemical phenotypes in patients treated for high-grade cervical intraepithelial neoplasia. BioMed Res Int 2013; 2013: 831907, doi: 10.1155/2013/831907.

28) SERESINI S, ORIGONI M, LILLO F, CAPUTO L, PAGANONI AM, VANTINI S, LONGHI R, TACCAGNI G, FERRARI A, DOGLIONI C, SECCHI P, PROTTI MP. IFN-γ produced by human papilloma virus-18 E6-specific CD4 + T cells predicts the clinical outcome after surgery in patients with high-grade cervical lesions. J Immunol 2007; 179: 7176-7183.

29) ORIGONI M, STEFANI C, DELL'ANTONIO G, CARMINATI G, PARMA M, CANDIANI M. Cervical Human Papillomavirus in transplanted Italian women: A long-term prospective follow-up study. J Clin Virol 2011; 51: 246-250.