

# Clinical Journal of Mycology

(formerly Mycology News) – February 2007 Volume 2, Edition 1

The Clinical Journal of Mycology (formerly Mycology News) is dedicated to the dissemination of information on the clinical use of mushroom nutrition to health care practitioners.

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# Evaluation of *Coriolus versicolor* Supplementation in HPV Patients

**Dr. J Silva Couto\*** Presented at the First Annual Monro Medical Lecture Series:  
HPV and Cervical Cancer: New Developments, September 23rd, 2006, Royal College of Physicians, London, United Kingdom

## Introduction

The treatment of HPV cervical lesions is presently undergoing re-evaluation. The traditional approach, which includes such treatment options as conventional surgery, laser surgery, cryosurgery and loop electrosurgical excision procedure (LEEP) are effective but may have reached the limits of their respective applications. For patients with high-grade squamous intraepithelial lesions (HSIL), cone biopsy (conization) is the method of treatment generally considered to be most effective.

For patients with low-grade squamous intraepithelial lesions (LSIL), the destruction of cervical lesions through the traditional treatment options has been modified to a more passive approach, commonly termed "wait and see" with increasing evidence that a women's immune system, if sufficiently strong, can control and possibly clear LSIL over time.

Vaccines have recently been developed against specific sub-groups of HPV virus. One has proved effective against HPV 16 and HPV 18, while another has demonstrated effect against a combination of 4 virus subtypes (HPV 6, 11, 16 and 18). Such vaccines are target sensitive to specific combinations of HPV viral proteins and both vaccines represent an important advance in HPV control.

However, neither of these vaccines is able to treat those already infected with HPV, or HPV subgroups beyond those listed, and it is recognized that substances with "immunostimulant" effect also have an important role to play in HPV control. What if we could find an immunostimulant substance able to induce resistance against all subtypes of the HPV virus?

The use of mushroom nutrition as an immunostimulant is an established practice in Asian cultures. *Coriolus versicolor* is one such mushroom, the biomass of which is a non-specific immunomodulator that has proved useful as adjunct nutrition to support the immune system in cancer patients undergoing chemotherapy or radiotherapy.

## Objectives

The aim of the present study was to evaluate the effect of *Coriolus versicolor* (biomass) supplementation in the evolution of cervical HPV lesions.

## Study Design

Patients were divided into two groups:

### – Patients with LSIL (CIN I and HPV)

The first group (LSIL) did not receive any conventional treatment. Fifty percent of the LSIL group received *Coriolus versicolor* supplementation for a period of one year (6 tablets a day – 3g/day). The remaining fifty percent received no supplementation or other medical treatment (control group).

### – Patients with HSIL (CIN II and CIN III)

The second group (HSIL) received cone biopsy, immediately after which fifty percent commenced *Coriolus versicolor* supplementation for a period of one year (6 tablets a day – 3g/day). The remaining fifty percent received no supplementation or other medical treatment following the cone biopsy (control group).

## Success Parameters

**The effect of *Coriolus versicolor* administration in the LSIL group was evaluated by measuring the degree of:**

- a) Reversion of the HPV positive stage (HPV+) to an HPV negative stage (HPV-)
- b) Cervical cytology normalization

**The effect of *Coriolus versicolor* supplementation in the HSIL group was evaluated by measuring the degree of:**

- a) Reversion of the HPV positive stage (HPV+) to a HPV negative stage (HPV-)
- b) The number of HSIL relapses

## Study Population

**54 patients with one year of follow-up were evaluated (35 HSIL and 19 LSIL).**

In addition to the 54 patients enrolled, 10 patients were excluded from the study for the following reasons:

- i) 2 patients who received a total hysterectomy
- ii) 2 patients where the first HPV tipification was inconclusive
- iii) 6 patients who had various side effects

The 19 LSIL patient group was then expanded to include an additional 18 LSIL patients who have not yet concluded one year of follow up.

## Material and Methods

As previously mentioned, the objective was to evaluate the effect of *Coriolus versicolor* supplementation in patients with LSIL who did not receive any conventional treatment and in HSIL patients who underwent cone biopsy. For this purpose, two groups of HPV patients were chosen (approximately 40 in each group).

### First (LSIL) Group

In the first group, patients with LSIL were all submitted to colposcopy, biopsy and HPV tipification at the first observation.

Patients' LSIL status was determined by cervical cytology exams (Pap smear tests) and reconfirmed through colposcopy and biopsy. Division of the LSIL patients into supplementation and control groups was aleatory (random).

Four months (120 days) after the first observation, all patients were once again evaluated through colposcopy and cervical cytology. At the same time, there was an evaluation of possible side effects from *Coriolus* supplementation.

When ending *Coriolus* supplementation after one year (365 days), all patients were examined for the third time by colposcopy, cervical cytology and HPV tipification.

A further evaluation will take place after 2 years (730 days), again with cervical cytology, colposcopy and HPV tipification. The efficacy of *Coriolus* supplementation was evaluated according to the evolution of HPV tipification from HPV+ to HPV- in the 2 groups, as well as the persistence of the cervical lesions over the course of the study period.

## Second (HSIL) Group

In the second group, those with HSIL, the patients brought their individual medical histories, which included cervical HSIL cytology and confirmation of biopsy outlining CIN II/III status. All patients underwent cone biopsy with laser or electro surgery (LEEP), at which time HPV tipification was conducted.

Half the patients, chosen by aleatory (random) selection, took *Coriolus* supplementation (3g/day) for 1 year following cone biopsy.

After four months patients' health status was evaluated and cervical cytology conducted. At the same time, possible side effects were evaluated.

The next observation was carried out on ending *Coriolus* supplementation after 1 year (365 days) when colposcopy, cervical cytology and HPV tipification were conducted.

The efficacy of *Coriolus* supplementation in the 2 groups was evaluated through a comparison of the evolution of HPV tipification from HPV+ to HPV- and the incidence of lesion relapses.

## Results (LSIL) Group

19 patients completed one year of follow-up. Of these 7 were initially positive for high risk HPV subtypes (HPV+ High Risk).

11 patients took *Coriolus* supplementation, while the other 8 patients received no supplementation (control), all were under clinical observation for 365 days.

Of the 7 patients who showed HPV+ High Risk tipification, 4 patients took *Coriolus* supplementation and 3 patients did not.

Of the 11 patients who took *Coriolus* supplementation over a year, only

# Viral Relationship between HPV and Cancer – Editors note

At least 80% of women are infected with HPV within 4 years of their becoming sexually active and HPV has been implicated in a range of cancers, including cervical, vaginal and vulval. Of these, cervical cancer is of particular importance as it is the fifth most common cancer in humans and the second most common cause of cancer death in women in the developed world. In some less developed countries, cervical cancer is the most common cause of cancer death. At least 90% of all cervical carcinomas are thought to be related to HPV infection, of which HPV-16 accounts for more than 50% of cases world-wide with HPV-18 accounting for a further 14%.<sup>(1)</sup>

It has been found that HPV subtypes 16 and 18 introduce two genes called E6 and E7, which code for proteins that inhibit p53 and Rb, two important tumour suppressor genes. The p53 gene product is involved in the regulation of apoptosis while Rb is responsible for halting the cell cycle at the G1-phase. When p53 is inhibited the normal process of cell death is obstructed and when Rb function is impaired, cell division is allowed to progress to the S-phase and complete mitosis, resulting in proliferation and hence neoplastic transformation.

In 2000, Ms. Marijke Pfeiffer (Centrum voor Integrale Geneeskunde, Amsterdam) noted when supplementing eight (8) HIV+ patients with *Coriolus versicolor* that one patient reverted to CIN-0 from a CIN-3 status over a 1 year period <sup>(2)</sup>. Noting Ms. Pfeiffer's work, Dr. Jean Monro (Breakspear Hospital) suggested that further research was needed to investigate this finding with a prospective protocol presented in Lisbon in May of 2001 <sup>(3)</sup>. In September of 2004, Dr. Couto (Portuguese Institute of Oncology-Coimbra) initiated clinical research outlined in his September 29th, 2006 presentation at the Royal College of Physicians <sup>(4)\*</sup>.

(1) Koutski L. "Papilloma virus and human cancers". Presented at the 37th Annual Meeting of the Infectious Diseases Society of America; Philadelphia, Pa; November 18-21, 1999. Session 61, S101.

(2) Pfeiffer, M. "The Clinical Use of *Coriolus versicolor* Supplementation in HIV+ Patients and its Impact on CD4 and Viral Load". Presented at the III International Symposium on Mushroom Nutrition in Milan on March 20th, 2001 in Milan, Italy. Reprinted in Mycology News 5 Vol 1, Edition 5 p 4.

(3) Monro J., "Cytokine Th1 to Th2 can be Reversed by *Coriolus*-Prospective Trial for HPV Control with *Coriolus*". Presented at the II International Symposium on Cancer and HPV in Lisbon, Portugal in May, 2001. Reprinted in Mycology News 10 Vol 1, Edition 10. p 4.

(4) Couto.C.J., Calisto R., Pereira da Silva D., "An Evaluation of the Efficacy of *Coriolus versicolor* in the Evolution of HPV Cervical Lesions". Presented at the Portuguese Society of Gynaecology 2-4 June 2006, Penha Longa-Sintra Portugal. Contact for Dr. Couto: jsilvacouto@sapo.pt. Copies of poster are available upon request from info@mycologyresearch.com

\* Dr. Silva Couto presented his research at the First Annual Monro Medical Lecture Series entitled HPV and Cervical Cancer: New Developments held at the Royal College of Physicians on September 23rd, 2006, a DVD of which is available from Margaret Schwartz of Breakspear Hospital. Email: MSchwartz@breakspearmedical.com

LSIL patients with <i>Coriolus versicolor</i> Supplementation	Evaluation after 1 year of supplementation
4 HPV+ High Risk 7 HPV- High Risk	4 HPV- High Risk 7 HPV- High Risk
LSIL patients without <i>Coriolus versicolor</i> Supplementation	Evaluation after 1 year of follow-up
3 HPV+ High Risk 5 HPV- High Risk	3 HPV+ High Risk 5 HPV- High Risk

1 still showed positive cervical cytology (LSIL) after a year of follow up.

Of the 8 patients who did not take any supplementation, 4 still showed positive cervical cytology (LSIL) after a year of follow-up.

The 4 patients who were HPV+ High Risk and received Coriolus supplementation all reverted to HPV- High Risk status after one year. Of the 3 HPV+ High Risk status patients who did not receive Coriolus supplementation, all remained HPV+ High Risk.

Analyzing the results according to cervical cytology evolution showed a persistence of LSIL cytology in patients who took Coriolus supplementation of 9% (1 out of 11 patients), while in LSIL patients who did not take any supplementation persistence was 50% (4 out of 8 patients).

In terms of the evolution of HPV tipification, it was concluded that all patients who took Coriolus supplementation (4 out of 4 patients) became negative for High Risk HPV (HPV- High Risk, 100% efficacy). In the 3 patients who did not take any supplementation, HPV tipification remained positive for High Risk subtypes in all, in other words a 100% persistence of HPV+ High Risk (3 out of 3 patients).

As previously stated, an additional 18 LSIL patients have now been added to the study, although they have not yet completed 1 year of follow up.

## HSIL Group

35 patients completed 1 year of follow up. 17 of these received Coriolus supplementation and 18 received no further treatment after cone biopsy. 28 of these patients had HPV+ High Risk tipification prior to cone biopsy, while 7 patients had HPV- High Risk tipification.

13 Patients of the HPV+ High Risk patients received Coriolus supplementation, while 15 received no further treatment after the initial cone biopsy.

**After 1 year the results for both groups were very similar:**

HSIL patients with <i>Coriolus versicolor</i> Supplementation	Evaluation after 1 year of supplementation
13 HPV+ High Risk	11 HPV- High Risk 2 HPV- High Risk
HSIL patients without <i>Coriolus versicolor</i> Supplementation	Evaluation after 1 year of follow-up
15 HPV- High Risk	13 HPV- High Risk 2 HPV+ High Risk

- None of the 35 patients was diagnosed with HSIL relapse.

- 1 of the 17 patients who took Coriolus supplementation showed LSIL cervical cytology after one year.

- 1 of the 18 patients who did not take the supplementation showed ASC-H cervical cytology after one year.

- Of the 13 patients with HPV+ High Risk who received Coriolus supplementation, 11 showed HPV- High Risk tipification and 2 were HPV+ High Risk after one year.

- Of the 15 HPV+ High Risk patients who did not receive any treatment besides cone biopsy, 13 were HPV- High Risk and 2 were HPV+ High Risk after 1 year.

## Side effects

6 patients stopped taking Coriolus supplementation due to reported side effects:

- 2 patients reported dizziness and headaches
- 2 patients reported gastric intolerance (vomiting and gastric pain)
- 1 patient reported diarrhoea
- 1 patient reported hirsutism (excessive hair growth on face and body)

No other side effects were reported and it was not necessary to take any kind of therapeutic action in the 6 cases above.

After stopping the Coriolus supplementation the symptoms did not persist.

## Conclusions

In the LSIL group of patients, although the results are preliminary as the study is continuing with additional patients, it can be concluded that Coriolus supplementation showed a high degree of success. when compared to the control group patients:



- Negative cervical cytology in 91% of cases compared to 50% of patients in the control group.
- HPV+ High Risk negation in 100% of cases compared to 0% of patients in the control group.

These results, which it should again be emphasized are preliminary, indicate positive benefits for LSIL patients from Coriolus supplementation.

In the second group of patients (HSIL), the conclusions are:

- Cone biopsy was highly effective at eradicating HPV+ High Risk as well as curing lesions (CIN).
- The efficiency of cone biopsy therapy reduces the potential benefits of any complementary supplementation.
- Only patients who remain HPV+ High Risk after surgery might benefit from complementary supplementation in the form of *Coriolus versicolor*.

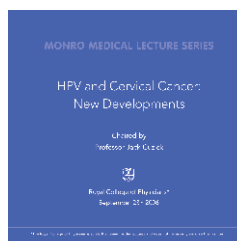
A DVD of the Monro Medical Lecture Series entitled 'HPV AND CERVICAL CANCER: NEW DEVELOPMENTS' is available from Margaret Schwartz of Breakspear Hospital.  
email: MSchwartz@breakspearmedical.com



\*Upon obtaining a medical degree from the University of Coimbra in 1973, Dr. Couto completed his internship in the Hospital of Coimbra (HUC), while teaching pharmacology at the University of Coimbra from 1975 to 1980. In 1978, Dr. Couto entered the Gynaecology Department of the Institute of Oncology (IPO) in Coimbra, where he became responsible for the cervical pathology unit and since 1995 has been head of the department. Dr. Silva Couto is based in the Gynaecology Department of the Portuguese Institute of Oncology (IPO), Coimbra, Portugal.  
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\*Dr. Silva Couto presented his research at the First Annual Monro Medical Lecture Series entitled **HPV and Cervical Cancer: New Developments** held at the Royal College of Physicians\* on September 23rd, 2006.

\*The Royal College of Physicians is solely the venue for the lectures and does not necessarily endorse their content.



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# Coriolus versicolor Supplementation in CIN-1 (LSIL) HPV Infection: Mode of Action

Professor Amin Karmali, Dr. Antonio Bugalho, Professor Tito H. Fernandes

## Introduction

Human papilloma viruses (HPV) are small, non-enveloped viruses containing a double-stranded DNA genome of around 8Kb. They are considered to be highly host-specific and infect epithelial cells.

There are some 100 strains of HPV in all with different genotypes, one small group of which has been identified as being responsible for certain types of tumours in different epithelia. This group of HPV is the number one cause of cervical cancer (carcinoma) (1). Other HPV strains cause genital warts and have led to HPV sometimes being called the wart virus or genital wart virus. However, the types of HPV that cause warts are not the types that cause cervical cancer.

There are 13 sub-types of HPV that are considered “high risk” for cervical cancer, including HPV 16, 18, 31 and 45. Of these, HPV 16 and HPV 18 are thought to be responsible for 70% of the cases of cervical cancer. High risk types can cause changes in the cells covering the cervix that make them more likely to become cancerous in time. If a patient has persistent or frequent infections with any of the ‘high risk’ types they are at risk of developing pre-cancerous cervical cells or cervical cancer.

Cervical cancer usually affects women between the ages of 35 and 55. Risk for cervical cancer seems to increase as a woman’s age at first sexual intercourse decreases and as the number of sexual partners increases. Failure to have a regular Pap test also increases risk (2).

Eighty-five (85) percent of cervical cancers are squamous cell carcinomas, which develop in the scaly, flat, skin like cells covering the outside of the cervix. The remaining 15% of cervical cancers develop from gland cells (adenocarcinomas) or a combination of cell types (adenosquamous carcinomas) (3).

## HSIL vs LSIL

Once diagnosed with HPV there may be a change in cervical epithelial from a normal stage (CIN-0) to either of two squamous cell types: high-grade squamous intraepithelial lesions (HSIL), or low-grade squamous intraepithelial lesions (LSIL). The degree of dysplasia is categorized according to five (5) categories:

CIN-0		Normal
CIN-1	LSIL	Minimal cervical dysplasia
CIN-2	HSIL	Moderate cervical dysplasia
CIN-3	HSIL	Severe cervical dysplasia
CIS	HSIL	Carcinoma <i>in situ</i>
	HSIL	Invasive Carcinoma

## CIN-2 / CIN-3 (HSIL HPV) Treatment

Usual treatment for HSIL patients (HSIL CIN-2 or HSIL CIN-3) involves a medical procedure removing lesions with a scalpel, laser therapy, or loop electrosurgical excision procedure. These surgical treatments preserve a women’s ability to continue to have children. As cervical cancer can recur, medical practitioners advise women to return for examinations and Pap tests every 3 months for the first year after surgery and every 6 months subsequently (4).

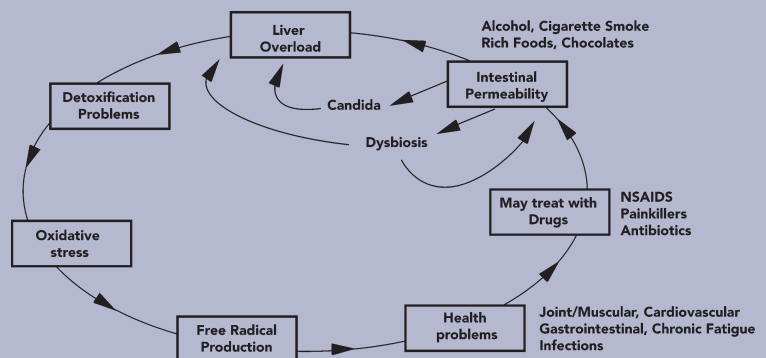
## CIS/Invasive Carcinoma (HSIL HPV) Treatment

If the cancerinogenic stage is advanced (HSIL Carcinoma in situ or HSIL Invasive Carcinoma), hysterectomy plus removal of adjacent structures and lymph nodes (radical hysterectomy) is necessary. Radiation therapy is usually also highly effective for treating advanced cervical cancer that has not spread beyond the pelvic region (5).

## CIN-1 (LSIL HPV) Treatment

The usual treatment for CIN-1 (LSIL-HPV) patients is one of “wait and see”. The prognosis of this situation is not as dangerous as with HSIL. In some cases, especially among women below the age of 35, their immune system is capable of “clearing” or keeping the virus under control. However, in women (and their sexual partners) over the age of 35, especially those who take oral contraceptives and smoke, their immune system is often too compromised to adjust to such chronic toxic overload (Fig 1). Consequently, when diagnosed with CIN-1 (LSIL-HPV) infection, such patients may need adjunct supplementation to support their immune system against progressive HPV infection. In chronic toxic overload, the body is in a vicious circle in which there is a

Fig-1- The vicious circle of chronic toxic overload



series of detoxification steps leading to oxidative stress, causing free radical production which results in health conditions generally solved with non-steroid anti-inflammatory (NSAIDS) and antibiotics. These drugs induce increased permeability of the intestinal wall (e.g. leaky gut) which is responsible for an overloaded liver causing detoxification problems, hence the vicious circle. (6)

It is essential that a CIN-1 (LSIL HPV) patient (and their sexual partner) faced with toxic overload finds an immune supporting and detoxifying agent that can absorb the free radicals, reduce their reliance on drugs to treat health problems caused by free radical production and simultaneously assists in the reduction of intestinal permeability.

## The Potential Role of Enzyme Therapy in CIN-1 (LSIL- HPV)

It has been known for over a century that some enzymes can be used in the prevention and even treatment of several clinical conditions and enzyme utilization in the form of mushroom nutrition supplementation may be useful for supporting the immune system in patients with toxic

overload and at risk of cancer<sup>(7) (8) (9) (10)</sup>.

In general, the oral administration of certain enzymes contributes to the efficacy of the following functions which can reduce chronic toxic overload in the organism:<sup>(10)</sup>

- **Restores balance to the internal environment**  
(such as neutral pH levels)
- **Removes toxic substances**
- **Helps re-establish intestinal bacterial equilibrium**
- **Strengthens the immune system**
- **Improves cell metabolism**

One example of how mushroom nutrition can improve the immune system was demonstrated using *Coriolus versicolor* (biomass) supplementation in thirty (30) Chronic Fatigue Syndrome (CFS) patients.<sup>(11)</sup> CFS patients with combinations of high antibody levels to EBV and/or HHV6 and CMV prior to *Coriolus* supplementation were given 6 tablets (3 g) per day of *Coriolus* supplementation for two weeks, followed by 3 tablets (1.5 g) per day for six weeks.

After supplementation, the following changes in immune parameters were noted<sup>(12)</sup>

**a) T cell activation (CD3 + CD26) in two-thirds of the patients.**

**b) NK cell (natural killer cell) increase of 35%.**

**c) Patients expressed a feeling of more energy based on the Fukuda quality of life scale.**

In summary, *Coriolus* supplementation significantly improved the immune profile of the CFS patients over 8 weeks of supplementation.

Other studies noted that *Coriolus* supplementation (9 tablets (4.5 g) per day) in thirteen (13) leaky gut syndrome (LGS) patients demonstrated beneficial improvement in ten (10) patients over twelve (12) weeks.<sup>(13)</sup>

Leaky gut syndrome (LGS) is the name given to a very common health disorder in which the basic organic defect is an intestinal lining which is more permeable than normal. The abnormally large spaces between the cells of the gut wall allow the entry of toxic material into the bloodstream that would, in healthier circumstances, be repelled and eliminated. The gut becomes leaky in the sense that bacteria, fungi, parasites and their toxins, undigested protein, fats and wastes normally not absorbed into the blood stream, pass through a damaged, hyper-permeable, or "leaky" gut. Leaky gut syndrome is found in patients with chronic toxic overload.

It is recognized that, while further studies are needed, LGS patients with low natural killer (NK) cell activity and low T-cell function could be potential candidates for *Coriolus* supplementation (4,5 g per day).<sup>(14)</sup>

More recent work with *Coriolus* supplementation for HPV patients has revealed that daily supplementation (3 g per day) of *Coriolus versicolor* (biomass) over one year had a significant impact on the risk factors for CIN-1 (LSIL HPV) patients. For example, in 11 CIN-1 (LSIL HPV) patients who took *Coriolus* supplementation:

**a) Only 1 patient had a positive cytology (LSIL) at the end of 1 year (remained at a CIN-1 status and did not return to CIN-0), a 91% success rate.**

**b) Of the 4 patients categorized as "high risk" (HPV+) at the beginning of the trial; at the end of the trial all 4 were categorized as "low risk" (HPV-).<sup>(15)</sup>**

Clinical work continues both to determine the optimum length of time for *Coriolus* supplementation in CIN-1 (LSIL-HPV) patients and to increase the number of patients in the trial.<sup>(16)</sup>

While these clinical results are considered encouraging for CIN-1 (LSIL HPV) patients, the question is "How does *Coriolus versicolor* assist the body's immune system?". In the following sections the impact of protein-bound polysaccharide complexes as well as the enzymatic action that characterizes *Coriolus versicolor* are reviewed.

## The Role of Protein-Bound Polysaccharide Complexes in *Coriolus versicolor*

Since the 1960's, extracts of *Coriolus versicolor*, known as PSK (Polysaccharide-K/Krestin) and PSP (Polysaccharide-Peptide), have been used in China and Japan as adjunct nutrition to improve the immune system of patients undergoing chemotherapy and/or radiotherapy.<sup>(17) (18)</sup>

In Japanese trials since 1970, PSK significantly extended survival at five years or beyond in cancers of the stomach, colon-rectum, oesophagus, nasopharyngeal and lung (non-small cell types), and in an HLA B40-positive breast cancer subset.<sup>(19)</sup> PSP was subjected to Phase II and Phase III trials in China and in double-blind trials significantly extended five-year survival in oesophageal cancer as well as significantly improving quality of life, providing substantial pain relief and enhancing immune status in 70-97 percent of patients with cancers of the stomach, oesophagus, lung, ovary and cervix.<sup>(20)</sup>

PSK and PSP boost immune cell production, ameliorate chemotherapy symptoms and enhance tumour infiltration by dendritic and cytotoxic cells. Their extremely high tolerability, proven benefits to survival and quality of life and compatibility with chemotherapy and radiotherapy makes them well suited for cancer management regimes in Japan and Hong Kong.<sup>(21)</sup>

The molecular mechanisms of biological response modification are not completely understood, although PSK and PSP are potent immunomodulators with specific activity for T-cells and for antigen-presenting cells such as monocytes and macrophages<sup>(22)</sup>.

A recent publication presents evidence regarding the presence of specific receptors for protein-bound polysaccharides in antigen-presenting cells (APC), B-cells and Helper T-cells.<sup>(9)</sup> The binding of these complexes to these cells triggers a variety of immunological responses including modulation of immunoglobulin production, Helper T-cell differentiation and function and APC - TH interaction. At the same time several reports have been published regarding the induction of apoptosis in several human cancer cell lines due to binding of protein-bound polysaccharides from mushroom strains.<sup>(23) (24) (25)</sup>

Pharmacologically active polysaccharides and protein-bound polysaccharides can be isolated from mushroom fruit-bodies, culture mycelium or culture broth of several basidiomycete strains such as *Coriolus versicolor*. Besides protein-bound polysaccharides, *Coriolus versicolor* contains several other biomolecules of clinical importance including enzymes and secondary metabolites (e.g. antibiotics and terpenes).

The biomass form of *Coriolus versicolor* contains protein-bound polysaccharides complexes and has demonstrated immune enhancement via increased NK cell activity.

## Enzyme and Secondary Metabolite Content in *Coriolus versicolor* (biomass)

Table I gives levels of SOD, cytochrome P-450, cytochrome P-450 reductase (NADPH dependent), laccase, peroxidase, protease, b-glucanase, protein-bound polysaccharides and secondary metabolites in 6 tablets (3 g) of *Coriolus versicolor* (biomass). The impact of gastric acid was simulated (in vitro) using the proteolytic enzymes pepsin and



**Table I**

Composition of 3g  
(6 tablets per day)  
of *Coriolus versicolor*

1. Pepsin (500IU/g biomass) at pH 2 for 30 min at 37°C in an incubator with orbital shaking

2. Trypsin (500IU/g biomass) at pH 7.6 for 30 min at 37°C in an incubator with orbital shaking.

<i>Coriolus versicolor</i> *	Absence of Proteolytic Enzymes	In presence of Pepsin	In presence of Trypsin
1 Protein (content)	103.8 mg	94.2 mg	99.6 mg
2 Reducing sugars	88.8 mg	87 mg	84.6 mg
3 Protein – bound polysaccharide	549 mg	483 mg	492.6 mg
4 Peroxidase activity	403.2 mU	362.4 mU	387 mU
5 Laccase activity	3129 mU	3069.6 mU	3210.6 mU
6 Glucoamylase/Beta – glucanase activity	41.4 U	30.0 U	37.2 U
7 Protease activity	35.4 U	30.0 U	31.2 U
8 Glucose 2 – Oxidase activity	297 mU	163.2 mU	270.0 mU
9 Cytochrome P – 450	3.06 nmoles	2.94 nmoles	3.12 nmoles
10 Cytochrome P - 450 reductase	71.4 mU	57.12 mU	66.6 mU
11 SOD activity	462.6 U	367.2 U	411 U
12 Secondary metabolites (Thrombin inhibitors)	59 %	54,2 %	52 %

trypsin in order to determine the degree of degradation due to enzyme action in the human intestinal tract.

Data presented in Table I demonstrates that in simulated digestive tract conditions (pepsin and trypsin) the level of enzyme and secondary metabolites decreased by a factor in the range of 15-20%<sup>(26)</sup>.

**Note: One enzyme unit (U) is defined as the amount of enzyme required to convert one micromole of substrate to product per minute under certain experimental conditions. One milli-enzyme unit (mU) is defined as the amount of enzyme required to convert one nanomole of substrate to product per minute under certain experimental conditions**

## Enzyme Effects in *Coriolus versicolor* Supplementation: Impact on the Immune System

As outlined in Table I, *Coriolus versicolor* biomass supplementation provides a natural route for enzyme administration. A summary of the mode of action of the aforementioned enzymes is given below:

### Enzymes Preventing Oxidative Stress

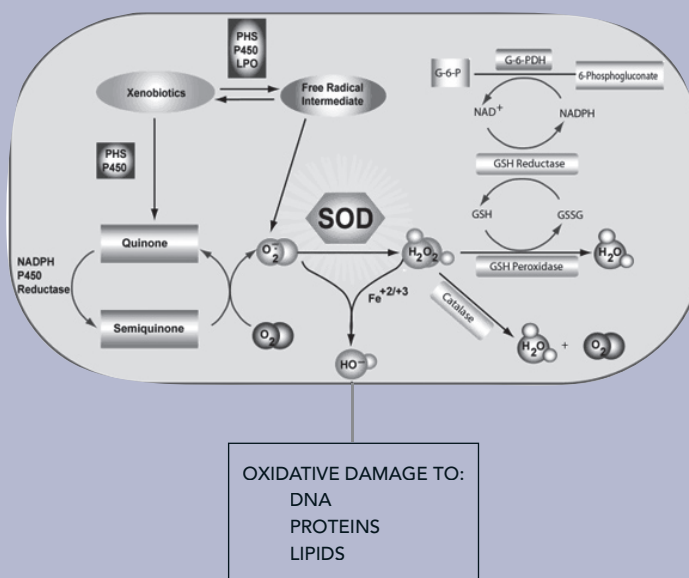
**a) Laccase (benzenediol: oxygen oxidoreductase; EC 1.10.3.2)** is present in its active form and catalyses the reduction of di-oxygen to water and the oxidation of a wide range of phenolic or related compounds. This enzyme also catalyses the oxidation of 3-hydroxyanthranilic acid (3-HAA) into cinnabarinic acid (CA) which is of great clinical interest since 3-HAA is produced in large quantities by interferon- $\gamma$  primed mononuclear phagocytes (7). Furthermore, 3-HAA has been shown to act as a powerful scavenger of reactive oxygen species. On the other hand, cinnabarinic acid (CA) is one of the major products of oxidation of 3-HAA, suggesting that laccase may prevent oxidative damage in mammalian tissues. In a similar manner, a mammalian protein, ceruloplasmin, like laccase, is a member of the blue copper oxidase class of enzymes which also catalyse the conversion of 3-HAA into CA.<sup>(27)</sup>

**b). SOD.** Cellular damage is induced by reactive oxygen species (ROS) which can be either free radicals (e.g. reactive anions containing oxygen atoms) or molecules containing oxygen atoms that either produce free radicals or are activated chemically by them. Examples of ROS include hydroxyl radicals, superoxide and hydrogen peroxide. There are several sources of ROS under physiological conditions such as aerobic respiration, sunlight, UV radiations, chemical reactions and metabolic processes (e.g. peroxisomal  $\beta$ -oxidation of fatty acids,

hepatic cytochrome P450 metabolism of xenobiotic compounds). However, under normal physiological conditions, ROS are degraded by the action of superoxide dismutase (SOD), catalase or glutathione (GSH) peroxidase. SOD catalyses the reduction of superoxide anions to hydrogen peroxide. SOD has been shown to play an important role in several clinical conditions, including Alzheimers, Parkinsons, cancer and aging. (Fig.2)<sup>(28) (29)</sup> SOD is a key agent in combating chronic toxic overload.

As NK cells are susceptible to reactive oxygen species (ROS) and lose their activity due to the effects of ROS. Cancer bearing hosts usually suffer from oxidative stress (OS) or toxic overload, and this causes the NK activity to decrease to a significantly lower level than normal. Superoxide dismutase (SOD) mimicking substances found in *Coriolus versicolor* and iron-chelating chlorine e6-Na (FeCNa), can restore the NK cell activity of cancer bearing hosts, when collaborating with catalase<sup>(30)</sup>. The relationship between the presence of SOD and effective NK cell activity in *Coriolus versicolor* biomass may explain the effectiveness of *Coriolus versicolor* biomass supplementation in increasing NK cell activity in Chronic Fatigue Syndrome patients.

**Fig.2** – The detoxification process under normal physiological conditions<sup>(31)</sup>





## Enzymes That Inhibit Cell Growth

**c) Protease activity.** The white-rot basidiomycete *Coriolus versicolor* manifests a significant amount of proteolytic activity. This fungus synthesises intracellular and extracellular proteases which are involved in the regulation of laccase and peroxidase activity in cultures of *Coriolus versicolor*. One protease specifically cleaves protein substrates (i.e. fibrinogen and casein) by hydrolysing certain peptide bonds. This enzyme is important for two main reasons. Firstly, it has high fibrinolytic activity and hence has potential as a therapeutic agent in the treatment of thrombosis. Secondly, this enzyme could be of use in protein sequencing due to its unique specificity. Proteolytic enzymes have been shown to degrade cancer cells as well as toxins whereas cellulases and b-glucanases strengthen the immune system and provide more ATP for cell energy.<sup>(32)</sup>

**d) Pyranose oxidase,** also known as glucose-2-oxidase (pyranose: oxygen 2-oxidoreductase; EC 1.1.3.10) catalyses the oxidation of several aldopyranoses producing hydrogen peroxide and 2-keto-D-glucose. Several species of basidiomycetes express this enzyme which also catalyses one-electron reduction of several different classes of xenobiotic compounds. This enzyme plays an important role in the clinical diagnosis of diabetes as well as in the production of fine chemicals and antibiotics (e.g. corticosterone).<sup>(33)</sup> The reaction product of glucose 2-oxidase catalysed reaction (i.e D-glucosone) has been found to exhibit anti-tumour activity (in vitro) against Ehrlich ascites tumour cells by inhibiting cell proliferation<sup>(34)</sup>.

## Enzymes Involved in Detoxification

**e) Peroxidases (EC 1.11.1.7).** These are a family of isoenzymes produced during secondary metabolism in white-rot basidiomycetes. These enzymes catalyse hydrogen peroxide-dependent one-electron oxidation of a wide range of phenolic and related compounds which result in the formation of aryl cation radicals. These radicals are converted non-enzymatically into several end-products. Currently, there is great interest in these enzymes because they can be used in the detoxification of a broad range of environmental pollutants including PCBs and dioxins.<sup>(35)</sup>

**f) Cytochrome P450.** The human race is constantly exposed to external toxins (e.g. polluted environment, cigarette smoke, alcohol, medication) as well as endogenous toxins (e.g. by-products from nutrient degradation, digestive tract bacterial waste products) which must undergo a detoxification process in the cell (Figure 2). Cytochrome P450 complex catalyses the oxidation and reduction reactions of several xenobiotic compounds i.e. chemotherapeutic agents. However, some reactions catalysed by cytochrome P450 also generate free radicals which may cause secondary cellular damage. In order to prevent such cell injury, an adequate supply of key antioxidant substances as well as free radical quenchers are required, such as reduced glutathione, superoxide dismutase (SOD), b-carotene and vitamin E.<sup>(36)(37)</sup> Cytochrome P450 is a key agent against chronic toxic overload.

## Secondary Metabolites

In addition to protein bound polysaccharides and enzymes, mushrooms have been shown to possess a large number of secondary metabolites (i.e. lectins, terpenoids, antibiotics and metal chelating agents), which may play an important role in the immune function of the host and hence could be of importance in immunotherapy of several disease states<sup>(38)</sup>.

## Conclusions

The immune profile for a CIN-1 (LSIL-HPV) patient (man or woman) over the age of 35, subject to chronic toxic overload, is extremely low. For this reason, the immunonutrition properties in *Coriolus versicolor* (biomass) supplementation should be considered as a clinical tool for

patients over the age of thirty-five (35) since supplementation at 3 g per day provides the delivery of:

1. Protein-bound polysaccharide complexes (beta-glucans) responsible for immune enhancement.

2. Enzymes that:

**a) prevent oxidative stress**

i) laccase activity

ii) superoxide dismutase (SOD) activity

**b) inhibit cell growth**

i) protease activity

ii) pyronase activity

**c) are involved in detoxification process**

i) peroxidase activity

ii) cytochrome P-450 activity

3. Secondary metabolites

The combined impact of these various modes of action could be responsible for improving the immune profile for CIN-1 (LSIL HPV) patients, thereby allowing their immune systems to “control” or to eliminate the HPV virus. However, it should be noted that *Coriolus* supplementation is not a substitute for medical procedures or medical products and further clinical work in a larger number of HPV patients is required to determine the full extent of its potential.

### Supplementation Schedule for CIN-1 (LSIL HPV) Patients

Weeks	Tablets per Day*	Tablets per Week	90 tab Bottles
1	6	42	
2	6	42	
3	6	42	
4	6	42	
5	6	42	
6	6	42	
7	6	42	
8	6	42	
9	6	42	
10	6	42	
11	6	42	
12	6	42	
13	6	42	
14	6	42	
15	6	42	
16	6	42	
	<b>TOTAL</b>	<b>672</b>	<b>8</b>

\*3 tablets in morning with breakfast and 3 tablets in evening with dinner

*Coriolus versicolor* biomass supplementation for CIN-1 (LSIL) HPV infection is used to support the immune system. The supplementation period is subject to regular cytological examination (Pap smears) to confirm return from a CIN-1 stage to a normal CIN-0 stage. Such cytological examination should be made on a regular basis (every six months) to monitor the condition. On average the cost per day of supplementation should be approximately £1.20 per day (\$2.35 or €1.75).

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\* Mushroom samples (in tablet form) were kindly provided by Mycology Research Laboratories Ltd. [www.mycologyresearch.com](http://www.mycologyresearch.com)

## Supplementation for Recurrent Herpes Simplex

As a doctor I am full of respect for the body's ability to fight illness and recover from trauma. In my professional capacity I like to think that with the aid of modern techniques for diagnosis and treatment I can facilitate the process of a person keeping healthy, or healing him or herself from illness.

Of all the organs and systems in the body that keep us going, one of the unsung heroes amongst these is the immune system. In recent years we have come to understand better how the cells of our immune system manage to recognise "foreigners" that may be trying to invade our bodies. The obvious foreigners are

prevent further attacks of herpes at some uncertain time in the future. The only way to prevent attacks is to take one of these medications in tablet form on a permanent basis.

In 2001 a female patient of mine came to see me at the beginning of her second pregnancy. She had started suffering from attacks of genital herpes five years previously and the attacks had become so frequent that she had been treated at St Mary's Hospital in London being prescribed acyclovir tablets, needing to take 400mg. twice per day to prevent attacks.

*I have suggested Coriolus versicolor as a treatment for recurrent Herpes to several patients with outstandingly good results and naturally this has fascinated me*

germs such as viruses, bacteria and parasites, and less obvious foreigners are any of our own body cells that have mutated to become abnormal and try to grow into cancers.

However, good as our immune systems are, they are not perfect. A particular chink in the protective armour of the immune system is illustrated by our susceptibility to some virus infections such as the Herpes virus, the wart (or Papilloma) virus, the Hepatitis B and C viruses and HIV. In this article I am going to describe my early experiences of treating Herpes simplex infection with a natural preparation made from a specific mushroom.

I am a traditional doctor working as a general practitioner in Portugal. While I accept that modern medicine has its limitations, I have always been comfortable with the efforts of good doctors to cure illness and relieve suffering. However, every now and then I have peered "over the other side" to look at what I can learn from the growing field of alternative medicine. For example, it has always been clear to me that acupuncture could be a useful technique – even if incomprehensible to my western mind.

One of the illnesses that modern medicine has not been able to cure is recurrent attacks of herpes simplex. These can occur either around the mouth as "cold sores" or in the genital region, tending to occur in almost exactly the same area of skin each time. There are orthodox medications which help to shorten attacks i.e. acyclovir as cream or tablets and now more modern alternatives. However, a treatment with these drugs does not

In 1998 she had fallen pregnant for the first time and had stopped the acyclovir because of the potential risks of exposing the foetus to the drug in utero. During this pregnancy she suffered 22 attacks of genital herpes which made the pregnancy a miserable experience.

I had recently been reading about the possible value of taking a preparation made from a mushroom called *Coriolus versicolor* in helping chronic viral infections. Having checked that it was safe to take Coriolus tablets when pregnant, I suggested to her that she could try taking it to see if it helped. She started taking three tablets twice per day reducing to three once per day.

To her and my amazement the effect was dramatic in that she only had a few minor attacks through the whole pregnancy, and when she felt an attack coming on she immediately doubled the dose to six tablets per day and this aborted the attack.

Furthermore, she continued to take Coriolus tablets three daily for another year or so, and had no more attacks for about two years. In the past two years she has had occasional attacks which she treats by taking three tablets twice per day which aborts the attack as long as she starts taking it quickly enough from the onset of symptoms.

Even though I had been very impressed with the dramatic benefits of taking Coriolus for my pregnant patient I still retained my instinctive scepticism about whether treating my patients with Coriolus would ever replace my prescription of orthodox

treatments. However, some months later one of my teenage sons started having trouble with oral herpes which seemed to be precipitated by the stress of doing end of school exams.

Initially I prescribed acyclovir cream which had little effect so I then prescribed acyclovir tablets. This was effective but as soon as an attack cleared up and he finished the tablets, another attack broke out. Once this had occurred two or three times I decided to prescribe Coriolus, starting with three tablets twice per day but then continuing with three tablets daily. He stopped getting herpes attacks.

A few months later he went abroad on a gap year project. He only took one container of Coriolus tablets with him, and so soon stopped taking them on a regular basis as he only had a small supply. However, when he did get an herpes attack he immediately started taking Coriolus and reported that it was at least as quickly effective as he had previously experienced taking high doses of acyclovir in treating the attack.

Since that time I have suggested *Coriolus versicolor* as a treatment for recurrent Herpes to several patients with outstandingly good results and naturally this has fascinated me. Research by Dr Jean Monro at Breakspear Hospital has suggested that the active ingredient is a polysaccharide from the cell wall of the mushroom which has a direct effect on white blood cells, specifically particular types of lymphocytes, to attack and kill cells which are harbouring herpes or other viruses.

There are claims that Coriolus can also be effective in preventing or as an adjunct to treating some forms of cancer. I have no experience in this area and at this stage do not intend to make any rash claims. However, there are grounds to consider that Coriolus might have a beneficial effect for two reasons.

Firstly, some forms of cancer are provoked by virus infections. Probably the outstanding example is cancer of the cervix which is the second commonest cause of cancer death in women. At least 90% of cases of cervix cancer are thought to result from infection of the cervix with the Human Papilloma Virus (HPV), especially with particular strains of HPV.

If taking Coriolus helped the body's immune cells to kill off the HPV infected cervix cells then the cancer might be either prevented or treated and there are trials underway in Portugal to test this theory in conjunction with orthodox treatments.

Secondly, the development of cancer in a person is to some extent the result of a failure of the immune system to recognise foreign, mutated, cancer cells which are attempting to grow. The reasons why this should happen are complex including genetic, dietary and toxic influences. Nevertheless, a person with a stronger immune system may have more success in fighting off invading cancer cells.

Support for the theory that an ingredient in some mushrooms may protect against cancer comes from a 15 year study of a large group of mushroom farmers in Japan who died from cancer much less frequently than a control group. Presumably they were protected by eating significant quantities of their mushrooms.<sup>(1)</sup>

I have been so impressed with the effect of Coriolus in treating patients who suffer from herpes simplex that I am going to

keep an open mind as to the possibility of treating other viral diseases with Coriolus or other mushroom preparations and also to the possibility of using these preparations as a supplement to traditional cancer treatments.

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#### Herpes Simplex Virus Supplementation Schedule with *Coriolus versicolor* biomass:

Weeks	Tablets per Day*	Tablets per Week	90 tab Bottles
1	6	42	
2	6	42	
3	3	21	
4	3	21	
5	3	21	
6	3	21	
7	3	21	
8	3	21	
	<b>TOTAL</b>	<b>210</b>	<b>3</b>

**\*3 tablets (500 mg) in morning and 3 tablets in evening with breakfast and with dinner in first two weeks; 3 tablets in morning with breakfast from week 3 to 8.**



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Mycology News is published by Aneid Press, a division of Aneid Lda

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ISSN 1646-6551