

Improvement of oral inflammation after treatment with hydrogen peroxide, *Coriolus versicolor*, and aromatic amounts of essential oils fixed on *Laetiporus sulphureus*

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Abstract

Background: Salivary calprotectin (sCPL) is a marker of neutrophil-induced inflammation, associated with periodontitis (PO) and systemic inflammatory diseases.

Objective: Assess whether sCPL level can be reduced with hydrogen peroxide, aromatic amounts of essential oils and *Coriolus versicolor*.

Methods: Data were collected from patients with severe PO and chronic systemic inflammation who consulted from January 2nd 2025 to June 2nd 2026. Salivary CPL was measured at the beginning and at the end of the observational study.

The patients were suggested to wash their mouth with hydrogen peroxide and to take aromatic amount of essential oils fixed on *Laetiporus sulphureus* in addition to *Coriolus versicolor* – a mushroom expected to control HPV and perhaps herpetic infections. The treatment lasted 6 to 12 months. Since PO is not expected to recover spontaneously, a decrease in sCPL above 100 IU/ml was considered an unexpected improvement. Pyruvate kinase M2 (PKM2) was measured in all patients with sCPL \geq 750 ui/ml.

Results: 40 patients were included. Among them 22 patients presented with sCPL \geq 750 ui/ml at inclusion. Salivary CPL level decreased in 31 patients (77.5%). It was unchanged in 6 patients and increased in 3 patients: two attributed to a drastic body weight increase and one to a flare of cholangitis. 9 patients were PKM2 positive at inclusion. Only two of them remained positive afterwards.

Conclusion: Hydrogen peroxide and aromatic amount of essential oils + *Coriolus versicolor* may decrease oral inflammation in patients with severe PO and chronic systemic disease.

Keywords: Calprotectin; hydrogen peroxide; Laetiporus; essential oils; Coriolus

List of abbreviations

CMV: cytomegalovirus

EBV: Epstein-Barr virus

FN: Fusobacterium nucleatum

HC: haemorrhagic colitis

HPV: human papillomavirus

HSV: herpes simplex virus

MASH: metabolic dysfunction-associated steatohepatitis

MS: multiple sclerosis

NSAIDS: nonsteroidal anti-inflammatory drugs

PG: Porphyromonas gingivalis

PKM2: Pyruvate kinase M2

PO: periodontitis

SCI: Silent chronic inflammation

sCLP: salivary calprotectin

Introduction

Saliva calprotectin (sCLP) is a simple and inexpensive marker to detect oral neutrophil-induced inflammation [1,2] and is increased in patients with periodontitis (PO) [3] which is associated with severe pathologies such as cancer [4], metabolic syndrome [5], psoriasis [6] as well as bone loss [7], or brain [8], cardiovascular [9], or joints inflammation [10], all of which requires active prevention.

Pyruvate kinase M2 (PKM2) has received increasing attention because of its role in tumour cell energy supply or proliferation, epithelial-mesenchymal transition, invasion and metastasis [11]. Its detection in saliva has been associated with colorectal polyps, dysplasia of the stomach or of the uterine cervix, as well as multiple sclerosis or Parkinson's disease [12]. The control of oral inflammation may perhaps enable to decrease silent chronic inflammation (SCI) and numerous severe diseases [13]. Hydrogen peroxide is an innocuous and efficacious agent to control oral anaerobic bacteria [14-16] and decrease the replication of many viruses with a lipid envelop such as SRAS-CoV-2, influenza or herpes simplex [17-20].

Coriolus versicolor is a mushroom with anti-human papillomavirus (HPV) and anti-herpetic properties [21-25]. Laetiporus sulphureus and some essential oils are effective against pathobionts and may have beneficial effects in PO or in Crohn's disease [26-28]. We investigated whether hydrogen peroxide use plus Coriolus versicolor and Laetiporus sulphureus+aromatic levels of essential oils could decrease sCPL and PKM2 levels and therefore help to control SCI.

Material and methods

This work is a descriptive retrospective epidemiological study. Data were collected during the normal course of routine gastroenterological consultations, from January 2nd 2025 to June 2nd 2026. There was no hypothesis testing before data collection, no data collection beyond that which is part of routine clinical practice, no scheduled data analysis before data collection. This retrospective analysis of Case Series cannot therefore be qualified as "research" and does not requires approval from ethics boards designed to protect humans involved in clinical research, according to the International Committee of Medical Journal Editors (ICMJE). French legislation does not require the consent of an Institutional Review Board in such epidemiological studies.

Inclusion criteria: All patients with a medical history of PO and chronic systemic disease, and who underwent a sCPL dosage at inclusion and 6 to 12 months afterwards were included. Patients signed a written consent for the possible use of the anonymized collected data.

Exclusion criteria: Lack of signed consent for possible retrospective epidemiological use of data; incomplete information on age, weight, height, sCLP at inclusion and 6 to 12 months afterwards.

Patients treated with strong anti-inflammatory treatment known to decrease TNF levels (such as methotrexate, steroids, anti-TNF antibodies) were excluded because they can induce a bias on the measurement of sCLP.

Dosage of salivary CLP: Bühlmann laboratories AG (Schönenbuch, Suisse) currently commercializes an ambulatory kit for the quantitative dosage of faecal CLP. We used this device for the dosage of salivary CLP according to the same protocol. We used 0.5 ml of saliva instead of 0.5 g of stools. The level of sCPL was assessed as stable when the change was lower the 100 ui/ml.

Detection of PKM2: Schebo biotech AG (Giessen, Germany) currently commercializes an ambulatory kit for a qualitative PKM2 test in faeces. We used this device for the dosage of salivary PKM2 according to the same protocol. We used 0.5 ml of saliva instead of 0.5 g of stools.

Statistics

The percentage of patients with $sCPL \geq 750$ ui/ml at inclusion was compared to the percentage at 6 to 12 months. The percentage of patients with detectable PKM2 at inclusion was compared to the percentage at 6 to 12 months. Comparisons of percentages used two-sample t-tests. The threshold of statistical significance was set to $p < 0.01$.

Limitations of the study

All inflammatory diseases were documented. No included patient was treated with corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs) or immunosuppressant therapy. No treatment was therefore expected to have a significant impact on oral inflammation. However, other confounding factors are possible. The lack of exclusion enables to conclude that no recruitment or selection bias is expected. However, all patients were Caucasian. Our conclusions may therefore be limited to a Caucasian population.

Results

This descriptive observational epidemiological study includes 40 patients. All patients present with severe PO and systemic chronic inflammation with Crohn, haemorrhagic colitis, anxiety/depression, rheumatoid arthritis, chronic pancreatitis, metabolic dysfunction-associated steatohepatitis (MASH), HPV infection, colonic polyps, cancer, psoriasis, vitiligo, Parkinson's disease, sarcoidosis, severe bronchiectasis/aspergillosis. None received anti-inflammatory treatment known to decrease TNF levels (such as methotrexate, steroids, anti-TNF antibodies or even NSAIDs) during the observation period. See (table 1). Patients were split into 2 groups at inclusion: Group 1 with $CPL \geq 750$ ui/ml and group with $CPL < 750$ ui/ml. Demographic data (gender, age, weight, height) were similar in both groups. Salivary CPL level was equal to 936 ui/ml \pm 94 in group 1 versus 600 ui/ml \pm 91 in group 2 ($p < 0.001$). PKM2 was positive in 41% of patients in group 1 versus 0% in group 2 ($p < 0.001$).

Table 1: Demographic data, sCPL level, PKM2 detection and medical history at inclusion

	Gender (% of female)	Age(years)	Weight(kg)	Height(cm)	sCPLui/ml	PKM2+(%)	Most relevant medical history(number of cases)
sCPL \geq 750 ui/ml 22 patients	36,4	56 \pm 12	60 \pm 10	166 \pm 8	936 \pm 94	9cases(41%)	Crohn/HC (4), MASH (3), HPV (3),MS (2), colonic polyps (2), cancer (2), psoriasis (2), vitiligo (2), Parkinson (1), sarcoidosis (1)
sCPL<750 ui/ml 18 patients	27,8	60 \pm 14	63 \pm 11	170 \pm 8	600 \pm 91	0 case(0%)	Anxiety/depression (4), MASH (2), psoriasis (2), RA (2), chronic pancreatitis (2), colonic polyps (1), cancer (1), Parkinson (1), sarcoidosis (1), HPV (1),Bronchiectasis/aspergillosis (1)
P value	>0.05	>0.05	>0.05	>0.05	<0.001	<0.001	NA

After 6 to 12 months of treatment (mouth cleaning with hydrogen peroxide + aromatic amounts of essential oils on a powder of *Laetiporus sulphureus* + *Coriolus versicolor*) the level of sCPL sharply decreased from 345 ui/ml \pm 161 in 30 patients. Nineteen patients switched from group 1 to group 2. Five patients presented with stable sCPL and the last 5 patients experienced an increased oral inflammation. Only 3 patients presented with a CPL level higher than 750 ui/ml at the end of the observational

study. Two of them had a medical of uncontrolled MASH and one patient had a medical history of breast cancer. Two patients presented with sCPL lower than 750 ui/ml despite and increased inflammation: one with a flare of cholangitis and one with uncontrolled MASH after a severe weight increase. Detection of PKM2 remained positive in two cases. One patient with psoriasis underwent colonoscopy and polyps were diagnosed. One patient with sarcoidosis was detected with cervix HPV infection. These two patients were PKM2+ at inclusion and after treatment. However, sCPL decreased substantially. No patients PKM2 negative at inclusion became positive afterwards. (Table 2).

Table 2: Follow-up after 6 months to 12 months of treatment Nineteen patients switched from group 1 to group 2.

	Evolution of oral CPL	PKM2 +	Most relevant medical history(number of cases)
sCPL \geq 750 Ui/ml 3 patients	One case: increase (536 to >1000)	No case(0%)	MASH with weight increase
	One case: stable (>1000 to >1000)		MASH without weight decrease
	One case: decrease (>1000 to 790)		Breast cancer (BCRA2+)
sCPL<750 Ui/ml 37 patients	30 patients with substantial sCPL decrease (-345ui/ml+/-161)2 patients PKM2+	2 cases(6.6%)	One with psoriasis*One with sarcoidosis**
	5 patients with stable sCPL	No case(0%)	All other patients (33)
	2 patients with sCPL increase		One with a flare of cholangitisOne with a flare of MASH

* colonic polyps were found afterwards

** Cervix HPV infection was found afterwards

Comparison of percentages at inclusion and at 6 to 12 months after treatment according to sCPL levels and PKM2 detection (see table 3) the percentage of patients with high sCPL levels decreased sharply after treatment: 55% of patients present with sCPL \geq 750 ui/ml at inclusion versus 7.5% after treatment ($p<0.001$). The percentage of patients with sCPL \geq 750 ui/ml and detectable PKM2 also dramatically decrease after treatment (22.5% versus 0%; $p<0.001$). All patients PKM2+ have high sCPL levels at inclusion. We concluded that the oral inflammation decreased sharply within 6 to 12 months. Since patients are their own control and that oral inflammation is not expected to spontaneously recover in case of severe PO, especially when associated with severe chronic systemic inflammation and when not treated with anti-inflammatory treatment, we hypothesise that the treatment participated to the improvement of these two biological local parameters.

Table 3: Comparison of percentages at inclusion and at 6 to 12 months after treatment according to sCPL levels and PKM2 detection

	At inclusion	At 6 to 12 months	P values
sCPL \geq 750Ui/ml	22 cases(55%)	3 cases(7.5%)	<0.001
sCPL<750Ui/ml	18 cases(45%)	37 cases(92.5%)	<0.01
sCPL \geq 750 Ui/ml and PKM2+	9 cases(22.5%)	No case(0%)	<0.001
sCPL<750 Ui/ml and PKM2+	No case(0%)	2 cases(5%)	NA*

Discussion

The control of oral chronic inflammation is the corner stone of anti-aging. Silent chronic inflammation (SCI) is implicated in numerous local or systemic diseases. SCI frequently involves the cardiovascular [29,30] or the central nervous system [31]. Cancers are largely attributable to at least 30 modifiable risk factors including nine infectious agents accounting for 10.2% of cases, just behind smoking (15.1%), however far ahead alcohol consumption (3.2%) [32]. We hypothesised that treatments of oral chronic infections - such as control of dysbiosis - may decrease oral SCI. The microbiota is defined as all the micro-organisms living on and in the human body. A pathogenic microbiota - dysbiosis - can harbour inappropriate bacteria, viruses, yeasts or parasites, leading to SCI.

Alterations in the gut microbiota frequently exist in patients with autoimmunity such as multiple sclerosis [33], lupus [34], Sjögren's disease [35], autoimmune thyroiditis [36], or inflammatory bowel diseases [37,38] and could contribute to the severity of inflammatory flare-ups. Decreased diversity of microbiota can also be considered as a kind of dysbiosis and is frequently detected in inflammatory diseases [39]. It is associated with inflammatory bowel disease [40,41] metabolic syndrome [42,43], as well as neutrophilic [44] or eosinophilic diseases [45]. Many diseases - such as Alzheimer's disease, Parkinson's disease or depression - are associated with small intestinal bacterial overgrowth, leaky gut syndrome and vagal-mediated inflammation currently classified as gut-brain axis disorders [46-48].

Epstein-Barr virus (EBV) and human papillomaviruses (HPV) are known to favour many types of cancers [49-52]. The association of viruses with bacteria triggers deleterious inflammation. For example, EBV may worsen *Helicobacter pylori*-induced SCI [53]. EBV, HPV and *Helicobacter pylori* are the most frequently reported infectious oncogenic pathogens [54,55]. The most frequent recognized types of chronic inflammation are visceral fat [56,57], chronic viral, or bacterial infections [58,59]. Periodontitis is associated with chronic viral and bacterial infections, and SCI [60,61], leading to many severe diseases [62-68]. It may concern up to 70% of US adults aged 65 years and older and is associated with more than 50 systemic inflammatory disorders and comorbidities, including cancers, neurodegenerative diseases or cardiovascular diseases [69]. Causal relationships are not yet established. However a bidirectional effect is currently admitted.

We previously established a list of infectious agents which may be implicated in SCI and that can be controlled by innocuous and inexpensive therapy [13], in extenso: HPV, EBV, CMV, HSV, *Fusobacterium nucleatum* (FN) and *Porphyromonas gingivalis* (PG). Oral calprotectin and PKM2 are relevant markers which belongs to a simple algorithm that was suggested in a previous work. Calprotectin is mainly synthesized by neutrophils [1] and is a simple and inexpensive marker to detect oral neutrophil-induced inflammation [2]. Saliva CLP is increased in patients with periodontitis [3]. Although the thresholds remain to be refined, the salivary CLP could already be recognized as a reliable marker of oral inflammation. Two previous observational studies identified 750 ui/ml as a possible threshold for severe inflammation and 450 ui/ml as a possible threshold for mild inflammation [2,12].

Pyruvate kinase M2 (PKM2) is a key enzyme for glycolysis and is closely related to tissue repair and regeneration. The switch to PKM2 modifies the glucose metabolism toward the Warburg effect which favours transformation, invasion, metastasis, and cell proliferation [70]. It has received increasing attention because of association with colorectal polyps, dysplasia of the stomach or of the uterine cervix, as well as multiple sclerosis or Parkinson's disease [71]. Pyruvate kinase M2 in stools is a recognized marker of dysplastic polyps of the colon-rectum [72]. Pyruvate kinase M2 can also be measured in the saliva and is strongly correlated with oral squamous cell carcinoma progression [73]. PKM2 detection is only qualitative. There is no published information on any quantitative approach in saliva. There is no evidence that a quantitative measure has a medical interest.

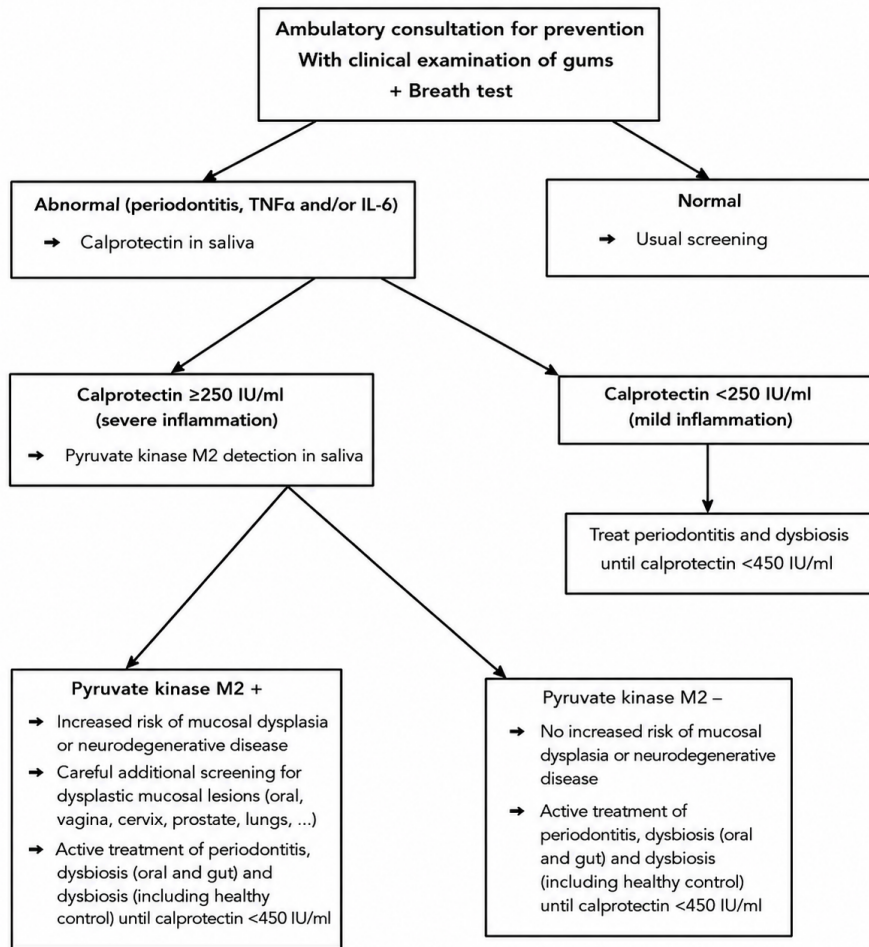


Figure 1: Proposed algorithm for the early detection of mucosal or brain chronic inflammation based on breath test, and calprotectine or pyruvate kinase M2 detection in saliva.

We previously suggested a practical ambulatory approach based on sCPL and PKM2 detection [13]. (See figure 1) PKM2 detection might be reserved for patients with high levels of sCPL in saliva. However, the detection of SCI is only of clinical interest when available and innocuous therapies exist. Infectious agents should therefore be controlled. Suggested integrative innocuous therapeutic approach to control oral and digestive viruses all targeted viruses (HPV, HSV1, CMV or EBV) are at least partly sensitive to *Coriolus versicolor*. *Coriolus versicolor* possesses well documented immunostimulating [74,75] and anti-viral properties [21-25]. Its efficacy against HPV is established [21]. The treatment valaciclovir + *Coriolus versicolor* is associated with a decreased risk of myeloma or lymphoma in asymptomatic monoclonal gammopathy [76]. *Coriolus versicolor* has also been reported to decrease Alzheimer's disease in a mouse model [77], and clear prion or intracellular bacteria [78,79]. It modulates Toll-Like Receptor4 [80], and inhibits many mycotoxins [81]. *Coriolus versicolor* is therefore expected to decrease the risk of many types of oral or gut-related inflammation.

Mouth cleaning against anaerobic bacteria

Fusobacterium nucleatum and *Porphyromonas gingivalis* are anaerobic bacteria implicated in periodontitis [82]. Periodontitis can be controlled by non-surgical treatment with mechanical mouth cleaning [83], photobiomodulation [84], or mouth wash with 1% hydrogen peroxide (H₂O₂). Hydrogen peroxide is also efficacious against SARS-COV-2 and herpes simplex [14-20]. *Laetiporus sulphureus* + aromatic amount of essential oils is known to decrease inflammation in Crohn disease [26] the benefit of *Laetiporus sulphureus* + aromatic amount of essential oils could be due to fibres, essentials oils against pathobionts or *Laetiporus sulphureus* it-self. The intake of fibres is beneficial in Crohn's disease [85]. An increased intake of organic vegetables is a

well-established recommended diet for body weight, cardiovascular diseases or cancer [86-88]. Organic foods may contain microbiota that may increase glycosides or polyphenols contents, and enrich the diversity of methanotrophs [89,90]. Aromatic amount of essential oils may decrease the amount of undesirable bacteria and increase diversity. For example, *Cinnamomum verum* or *Origanum vulgare* could be efficacious against FN and periodontitis at very low dose [91,92].

Please note that encouraging results have been reported after the improvement of microbiota due to faecal graft in patients with melanoma, renal carcinoma or non-small cell lung cancer. These improvements are attributed to the destruction of inappropriate bacteria rather than actual implantation of beneficial germs [93,94]. *Laetiporus sulphureus* exerts multi-targeted protection against colitis by reinforcing epithelial barrier function, attenuating inflammation, and reshaping gut microbial ecology [95]. It possesses antioxidant and antimicrobial properties [96]. Synergies between all these agents are plausible and the actual beneficial mechanism could be tricky to be unravelled in detail. Since they all are innocuous, inexpensive and considered as food ingredients, we decided to use all of them at once rather than introduce them step by step.

The results of this observational study

We previously suggested three actions to control SCI [13]. Firstly, mechanical mouth cleaning with hydrogen peroxide mouth wash, *Coriolus versicolor* and aromatic amounts of essential oils, especially *Origanum vulgare compactum*. Secondly, decrease visceral fat with a specific diet, *Grifola frondosa*, *Laetiporus sulphureus* and aromatic amount of essential oils [97]. Thirdly, increase organic food (especially green vegetables and fibres), which probably contains endobiota and natural polyphenols. Please note that food complements do not contain endobiota and cannot increase microbiota diversity.

This observational study aims to evaluate the impact of the first step on sCLP. Therefore, we did not ask the patients to modify their diet. Please note that *Laetiporus sulphureus* [95] or *Coriolus versicolor* [98] may however reshape gut microbial community. This study confirms that this action which mainly focused of oral viral/bacterial infections is particularly useful regarding oral and perhaps systemic inflammation. Three failures out of four to reduce sCLP levels were related to weight increase. This finding foreshadows that diet enabling weight and visceral fat loss may further improve the global results.

Clinical implications and future directions

This work enables to suggest the hypothesis that hydrogen peroxide + aromatic amounts of essential oils fixed on *Laetiporus sulphureus* + *Coriolus versicolor* decreases oral inflammation. We suggest that diet to decrease visceral fat could be added when needed. Further studies should be performed firstly to confirm this positive effect on oral inflammation and secondly to observe consequences on systemic diseases and on their late complications such as cancer, neurodegenerative or cardiovascular diseases.

It is possible that the relevance of the third step – which emphasizes on the diversity of the gut microbiota – will be significantly reduced after the implementation of mouth cleaning and after the reduction of visceral fat. However, one should keep in mind that mouth cleaning and weight control could generate diversity of the gut microbiota by them-selves. This study provides a new argument in favour of the determination of sCLP to evaluate and follow SCI in patients with PO and systemic chronic diseases. The flow chart depicted in figure 1 is not time consuming, and appears reliable to detect SCI which may spread to almost all organs.

Limitations of the study

The absence of randomization prevents any conclusion as to a causal relationship. However, the patients were their own control, which reduces the risk of bias. All patients were Caucasian which may limit our conclusion to this population. Not all co-

morbidities or medications are provided in this article. However, all causes of severe inflammation are provided. No patient was treated with corticosteroids, NSAIDs or immunosuppressant therapy (such as TNF antagonists). Therefore, no treatment is expected to have a significant impact on oral inflammation.

Conclusion

We hypothesise that mouth cleaning with hydrogen peroxide, aromatic amount of essential oils fixed on *Laetiporus sulphureus* and *Coriolus versicolor* may decrease sCPL levels and therefore oral inflammation. Decrease visceral fat and liver steatosis could improve results. We therefore suggest to measure sCPL in all patients with PO, especially in those with systemic inflammatory diseases. The lack of early detection of oral inflammation could mean a loss of opportunity for a large part of the population; especially if we consider that oral inflammation may be easily controlled by inexpensive and innocuous therapies. Further studies are required to refine the threshold levels for sCPL and assess the value of reducing salivary CLP on gut dysbiosis, stomach emptying and body weight, dysplastic mucosal lesions, some cancers, osteoporosis, alveolar bone loss, anxiety-depression episodes, etc.

Conflict of interest

DFN sarl. Farming business. Production of organic natural flavors

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