



# **Health resort and human immune response**

*How balneology can protect  
and improve our health*

**Editor: Stefano Masiero**

**The University of Padua (Italy)**  
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An Initiative of the World Federation of Hydrotherapy and Climatotherapy

FEMTEC , NGO in official relations with World Health Organization



## Preamble

Water is the most valuable resource for life. It accounts for 70% of the weight of the human body and is the “cradle” for its growth since conception. It is the basic element to preserve and promote man’s wellbeing and health in all respects and at all stages in life. Water is also closely connected with civilization, as evidenced and celebrated in literature, architecture, and art. The clinical and scientific evaluation of its health properties – already well-known and appreciated in the Roman era and constituting the foundation for the hydrological culture and its different physico-chemical components and usage modes – is at the basis of the balneology, still an actually discipline despite the great technological progress of modern medicine.

Balneology is a medical discipline included by WHO\* in the field of complementary and integrated medicine and uses natural methods of treatment, prevention and rehabilitation. Researchers and scientists from the most advanced Countries are committed to understanding the mechanisms of action of balneotherapy both at the level of basic science and clinical. Institutions of great prestige such as Afreth (Association française pour la Recherche thermale) and FoRST (Italian Foundation for Thermal researches) are strongly engaged with FEMTEC in promoting research according to the most modern methods of investigation.

The recent COVID-19 pandemic that has deeply affected the health conditions of millions of people and radically changed our lifestyles, has also posed important scientific dilemmas, which are only partially clarified today. One of the aspects that have emerged in the prevention, therapy and rehabilitation from Covid-19 infection is the important role played by the immune system, so we asked ourselves whether there were the obvious scientific assumptions to hypothesize the important role that the use of proper balneotherapy could have in improving the immune system of the people.

Being conscious of the importance of appropriate information of both specialists and consumers, FEMTEC is releasing this “initiative document” to provide reliable scientific data based on specific methodological criteria, as well as on the role of balneotherapy in man’s health and wellbeing and as a crucial component for the global promotion and development of general health and wellbeing.

The Authors, dr. Maria Chiara Maccarone, dr. Giacomo Magro, and dr. Anna Scanu, coordinated by Prof. Stefano Masiero, Director of the Department of Rehabilitation at the University of Padua (Italy) and President of the Femtec Commission for Physical Therapy, have carried out an accurate research and critical analysis of the existing literature on the subject and present a general picture of undoubted positive interest that invites not only to confirm the important role of balneotherapy in the prevention of diseases but opens up interesting further fields of research.

Founded in 1937, FEMTEC (the World Federation of Hydrotherapy and Climatotherapy ([www.femteconline.org](http://www.femteconline.org))) is one of the main representatives of Thermal Medicine and Hydrotherapy and coordinates the activity of the sector’s national Institutions in over 30 countries. FEMTEC entertains official relations with the World Health Organization (WHO), with which it cooperates to develop and implement programmes for the scientific use of Complementary Medicines. The Federation cooperates with the ISMH (International Society of Medical Hydrology), as well as with Universities and Ministries of Health in several Member States.

Before concluding, we wish to express our appreciation to those that participated to the drafting of this paper. In particular acknowledge our colleagues Stefano Masiero, Maria Chiara Maccarone, Giacomo Magro, and Anna Scanu for their dedication and competence.

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in diffusing the paper. The opinions expressed herein are those of the Authors and do not necessarily reflect those of Foundation.

Prof. Umberto Solimene President FEMTEC

\*1 WHO (2000). General guidelines for methodologies on research and evaluation of traditional medicine. Geneva, <http://apps.who.int/medicinedocs/pdf/whozip42e/whozip42e.pdf>. In its latest paper Traditional Medicine Strategy: 2014-2023 the WHO confirms the need to improve knowledge

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## Introduction

### The human immune system

We are continually exposed to organisms that are inhaled, swallowed, or inhabit our skin and mucous membranes. Whether these organisms penetrate and cause disease is a result of both the pathogenicity of the organisms (the virulence factors) and the integrity of host defence mechanisms represented by the immune response. The immune system is a complex network of lymphoid organs, numerous cell types, humoral factors, and cytokines. The essential function of the immune system is highlighted when it goes wrong: severe infections and tumours are linked to immunodeficiency, whereas immune system overactivity leads to allergic and autoimmune disease.

Two major subsystems compose the immune system: the innate immune system and the adaptive immune system. Both subsystems use humoral immunity and cell-mediated immunity to perform their functions.

### Immune system influence factors

#### *Age*

Immune variation among individuals is largely due to differences in age. People have less robust immune responses as they age, and the elderly are more prone to infection.

One major cause of age-related immune deficiencies is thymic involution, the shrinking of the thymus gland that begins at birth, at a rate of about three percent tissue loss per year, and continues until 35–45 years of age, when the rate declines to about one percent loss per year for the rest of the life. There is evidence of a loss of thymic epithelial cells according to the decreasing expression of the FOXP1 gene with age. It is also known that thymic involution can be altered by hormone levels, in particular sex hormones such as estrogen and testosterone enhance involution. The hormonal changes in pregnant women cause a temporary thymic involution. So the progressive decline in hormone levels with advancing age is believed to be partially responsible for the weakened immune response found in older individuals.

The age-related decline in immune function is also linked to the decrease in vitamin D levels in the elderly.

#### *Sex*

Sex is a biological variable that affects the functions of the immune system, with sex differences in both innate and adaptive immune responses. Sex differences in immune responses change throughout life and are influenced by both the age and the reproductive status of the subject. Sex chromosome genes and sex hormones, including oestrogens, progesterone and androgens, contribute to the differential regulation of immune responses between the sexes.

Sex differences in immune responses result in differential susceptibility of males and females to autoimmune diseases, malignancies and infectious diseases.

### *Hormonal regulation*

As already said, hormones can have a peculiar role in the immune responses regulation. In particular, they can act as immunomodulators, altering the sensitivity of the immune system. For example, female sex hormones are known to be immunostimulant of both the adaptive response and the innate response. On the other hand, male sex hormones, such as testosterone, appear to be immunosuppressive. Other hormones are also thought to be able to regulate the immune system, especially prolactin, growth hormone and vitamin D.

### *Sleep and rest*

The immune system is affected by sleep and rest; indeed, sleep deprivation has been shown to be harmful to immune function. When suffering from sleep deprivation, active vaccinations can have a reduction in their effects causing a reduced production of antibodies and a less efficient immune response, compared to what would be observed in a well-rested individual. In addition, proteins such as NFIL3 have been shown to be closely related to both the differentiation of T lymphocytes and the circadian rhythms and they can be influenced by sleep deprivation.

Disruption of the circadian rhythm has also been shown to have strong effects on the immune function affecting both innate and adaptive immunity. During the initial phase of sleep, a sudden drop in blood levels of cortisol, adrenaline and norepinephrine induce an increase in blood levels of leptin hormones, pituitary growth hormone and prolactin. These signals induce a pro-inflammatory state, due to the production of interleukin 1, interleukin-12, TNF-alpha and gamma interferon cytokines. These cytokines stimulate the activation of immune cells, their proliferation and differentiation. During waking periods, natural killer cells and cytotoxic T lymphocytes have a peak of activity obtaining an effective response against any pathogens. There are two theories that try to explain why the pro-inflammatory state is reserved for sleep time. The first one considers that if inflammation occurs during the waking periods, it would cause serious cognitive and physical impairments, while the second one considers the presence of melatonin during sleep periods that could actively counteract the production of free radicals.

### *Stress*

Short-term (lasting for minutes to hours) stress experienced during immune activation enhances innate and adaptive immune responses through changes in dendritic cell, neutrophil, macrophage, and lymphocyte trafficking, maturation, and function as well as in local and systemic production of cytokines. In contrast, long-term stress suppresses or dysregulates innate and adaptive immune responses by altering the Type 1-Type 2 cytokine balance, inducing low-grade chronic inflammation, and suppressing the level and the function of immunoprotective cells.

### *Genetic variation*

Genome-wide association studies of 166 immunophenotypes identified 15 loci that could be involved in the development of diseases linked to a dysregulated immune response. The parameters of innate cells are more strongly controlled by genetic variation than those of adaptive cells, which are driven mainly by environmental exposure.

### *Other factors*

Environmental factors, including nutrition status and the composition of the microbiome, also alter the development and the function of the immune system. Too high nutritional supplies are related to diseases such as diabetes and obesity that are known to affect immune function. Even a bad diet, as well as some deficiencies in minerals and specific nutritional elements, can cause a compromised immune response. Fetal malnutrition can cause permanent damage to the immune system. On the other hand, foods rich in certain fatty acids can be promoters of a healthy immune system.

Smoking and asymptomatic cytomegalovirus infection, which affects 35% of the population, can also have a major impact in blood cell composition. This may explain why smokers and people infected by this virus can be more prone to infections.

## Health resort medicine

Core elements of health resort medicine are balneotherapy, hydrotherapy, and climatotherapy. Health resort medicine can be used not only for wellness concepts, but also for health promotion, prevention, treatment, and rehabilitation based on the healing effects of water.

Balneotherapy (BT) is one of the most commonly used complementary therapies that generally employs mineral and thermal waters from natural springs, medical peloids (mud), and natural gases through different modalities (such as bathing, drinking, inhalation, etc.). Other interventions frequently used in health resorts are massage, exercise, sauna, Turkish bath, physical therapies, kinesiotherapy, pharmacotherapy, psychotherapy, nutrition, health education and cognitive behavioural therapies, relaxation therapies, and other complementary therapies. Additionally, health-promotion, cultural and social activities as well as sports can be part of health resort medicine.

## Thermal techniques and biological effects

Thermal water has peculiar properties that can be exploited through different techniques applied in health resort medicine.

### *Balneotherapy*

BT has been employed for the treatment of different pathological conditions (dermatological, rheumatological, gastroenterological conditions, pulmonary diseases, cardiovascular, gynaecological, metabolic, neurological, psychiatric, and endocrine disorders). A large number of clinical studies have reported the beneficial effects of this approach for the prevention, treatment, and rehabilitation of



various rheumatic disorders, such as osteoarthritis, low back pain, rheumatoid arthritis, and fibromyalgia with positive outcomes on pain, function, quality of life and cost/effectiveness. Also on dermatological conditions, such as psoriasis and atopic or contact dermatitis, growing evidence has disclosed the potential therapeutic properties of BT.

The beneficial effects of BT are related to the physical and chemical properties of mineral-rich waters, including temperature, salt composition and concentrations, buoyancy, viscosity, and hydrostatic pressure.

Regarding temperature, water, having a high specific heat and a low conductivity coefficient, has a great capacity to retain heat and to transfer it progressively to the patients. Warm baths can also cause a sense of relaxation and contribute to muscles elasticity. The water salt content, boosting heat through the direct relation of the specific weight with the thermal capacity and the heat retention capacity and boosting hydromechanical action, also contribute to the positive effects of thermal water. Sulphurous water, salt bromine, iodine water and radioactive water are commonly used for rehabilitation treatment in musculoskeletal diseases. Other common BT techniques include: hydromassage, hydropressotherapy (jets of hot/cold thermal water manually directed to the whole body), and cardiovascular circuits (exploiting hot thermal water and cold water).

#### *Mud or peloid therapy*

Peloid is natural mud, used for packs or baths, obtained by mixing water (thermal, sea or lake/river) with inorganic, organic or mixed materials, derived from geological or biological processes. Mud is applied to the skin at a temperature of 45-50°C. Patients are subsequently covered with sheets, cellophane and blankets to reduce heat dispersion. The duration of each application is 15-20 minutes. At the end of the session, patients receive a cleansing bath or a shower with normal or thermal water. Subsequently, patients go to a cabin where they rest for 30-60 minutes.

The biological and therapeutic effects of muds are due to the anti-inflammatory, analgesic, myorelaxant, and trophic effects.

Mud baths are especially used for osteoarthritis, ankylosing spondylitis, chronic lumbar pain, peri-arthritis, extra-articular rheumatism, and tendonitis. They are also suitable for peripheral vessel disease, skin conditions (ulcers and skin dystrophy), and gynaecological issues.

#### *Hydropinotherapy*

This treatment involves drinking mineral water at regular intervals throughout the day. Hydropinotherapy is recommended for treating urinary tract disorders, or intestinal diseases and in general for gastroenteric problems.

#### *Inhalation treatment*

Thermal water can also be useful to treat chronic states of inflammation and irritation of the upper and lower respiratory tracts through inhalations.

#### *Irrigation*

Irrigations consist of bringing water into contact with the mucus membranes of open cavities in the body (nasal irrigations, indicated for catarrhal and atrophic rhinitis and ozena; oral cavity irrigations, indicated for periodontitis, chronic dental disease and pharynx infections; vaginal irrigations to treat chronic gynaecological inflammation; intestinal irrigations to treat colon diseases).

## Balneotherapy and the immune system

In this historical moment in which the world is facing COVID-19 pandemic, it is interesting to know whether health resort medicine, and in particular mud and baths applications, can also affect the immune system. In recent years, there has been an increased interest in the use of preclinical models (in vitro studies on human or animal samples) to investigate the biological effects of BT on inflammation and immunity. Many mineral-rich waters have been shown to have effects on the immune system and recent findings suggest that BT may improve the efficacy of immune response even if the mechanisms through which immersion in mineral-rich water or mud therapy may be useful to improve human immune function are still not completely understood. Recently, also clinical trials and RCTs have been developed to study the effects of BT on human immune system.

The biological mechanisms by which immersion in mineral-rich water and mud applications alleviate symptoms of several pathologies are still not completely understood. However, in recent years, immunological mechanisms of effectiveness have been studied, showing that anti-inflammatory effects could be responsible of BT clinical benefits.

## Aims of the project

The objective of this project is to summarize the current available information about the effects of thermal mineral waters or of their organic and inorganic components on the immune response.

## Materials and Methods

Search strategy. Studies were found by screening PubMed and Google Scholar database from 1997 up to June 2020. As keywords we use spa therapy, health resort medicine, balneotherapy, mud therapy, immune response, immunity, immune system.

Study selection. Studies were eligible if they were in vitro research on human or animal samples, randomized controlled trials (RCTs) or clinical trials; health resort medicine had to be the intervention under study and had to be compared with another intervention or with no intervention.

## STATUS OF HEALTH RESORT MEDICINE AND IMMUNE SYSTEM EVIDENCE

### In vitro studies on human samples evaluating the beneficial effects of BT on immune response in skin diseases

Most of the in vitro studies on human samples evaluating the effects of thermal waters on the immune response in dermatology focused on psoriasis.

Psoriasis is considered a genetically programmed, immune-mediated, inflammatory disease, in which intralesional T lymphocytes induce keratinocytes to proliferate and perpetuate the disease process. The

interleukins IL-17 and IL-22 produced by Th1/Th17 lymphocytes stimulate IL-8 secretion by keratinocytes, and this represent a key event in the pathogenesis of the disease. Sulfur is able to penetrate the skin, and sulfur-rich waters used in balneotherapy have been suggested to be effective in the treatment of psoriasis.

Evaluating the effects of thermal water rich in NaHS, [Gobbi et al. \(2009\)](#) and [Mirandola et al. \(2011\)](#) demonstrated that a cell culture of human psoriatic keratinocytes treated with it can show a reduction in the inflammation characterising psoriatic lesions, in particular mediated by a reduction in IL-8, IL-17 and IL-22.

Since skin psoriatic manifestations are thought to be angiogenesis-dependent, effects on the expression and release of VEGF-A were studied by [Chiarini et al. \(2006\)](#) using Comano’s thermal water, rich in sodium, calcium and bicarbonate. They demonstrated, evaluating cultured human lesional keratinocytes isolated from skin biopsies performed in 9 patients exposed for 11 days to thermal water, that BT with Comano’s thermal water, used for psoriasis and other skin disorders, interferes with VEGF-A isoform expression and secretion by the psoriatic keratinocytes. These effects would reduce all VEGF-A-mediated angiogenic, vessel permeabilising, and chemotactic effects, explaining the beneficial actions on the clinical manifestations of psoriasis.

In another study by [Chiarini et al. \(2006\)](#) confluent cultures of IL-6-hypersecreting keratinocytes isolated from 6 psoriatic patients were exposed for 11-15 days to Comano’s thermal water. After the exposure, the intracellular levels and the secretion rates of IL-6 were drastically reduced, and also the expression of cytokeratin-16 (CK-16), a marker associated with keratinocyte psoriatic phenotype, was persistently down-regulated. Hence, Comano’s water balneotherapy may beneficially affect the clinical manifestations of psoriasis via an attenuation of the local deregulation of several cytokines/chemokines, including IL-6 and VEGF-A isoforms.

Finally, [Dal Pra et al. in 2007](#) using the same water showed a reduction in cultured human primary epidermal keratinocytes in the intracellular and secretion levels of IL-8 and TNF- $\alpha$ , pro-inflammatory cytokines overexpressed in the psoriatic lesions, with a consequent reduction in the inflammation psoriatic events.

[Karagülle et al. in 2018](#) evaluating human keratinocyte cell lines model of psoriasis and rosacea treated with Bursa and Bolu thermal mineral waters showed a reduction of inflammation and neo-angiogenic phenomena linked to skin disease manifestations. In particular, a reduction in IL-1 $\alpha$ , TNF- $\alpha$ , and VEGF gene expression was found.

[Lee et al. in 2012](#) evaluated the effect of spa spring water from Yong-gung oncheon on cells related to the skin immune reactions. The treatment with thermal water decreased in the human keratinocyte cell line the expression of proinflammatory cytokines under TLR stimulation. In particular, they observed a significant inhibitory effect on the secretion of IL-6, IL-8, IL-1 $\alpha$ , TNF $\alpha$ , and GM-CSF from keratinocytes. In addition, spa spring water attenuated the differentiation process of subsets of T helper cells, i.e. Th1, Th2 and Th17 cells (related to the autoimmunity or to inflammatory skin diseases, such as psoriasis or atopic dermatitis) after 1, 4, 10, or 24 h of treatment. Thermal water from Yong-gung oncheon induced Foxp3<sup>+</sup> T<sub>reg</sub> cell differentiation *in vitro*, implying that the immunomodulatory effect also includes T<sub>reg</sub> cell-induced immune suppressive effects.

All the results of these studies are reported in Table I.

Authors	Journal	Pathology	Experimental model	Treatment	Mineral water or inorganic or	Biochemical parameters	Results
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					organic components		
Gobbi et al. 2009	Lab Investig	Psoriasis		30-min preincubation with MAPK/ERK inhibitors (10–30 µM) + NaHS (400 mM) dissolved in the culture medium, for 6, 12, 18, 24 h	NaHS	IL-8, IL-17, IL-22; cell proliferation and adhesion; MAPK/ERK signaling phosphorylation	Reduced IL-8, IL-17, IL-22 secretion, adhesion molecules expression, and MAPK/ERK phosphorylation Reduction of inflammation events typical of psoriatic lesions
Mirandola et al. 2011	Lab Investig	Psoriasis	Normal skin-derived immortalized human keratinocytes	30-min preincubation with MAPK/ERK inhibitors (10–30 µM) + NaHS (400 mM) dissolved in the culture medium, for 6, 12, 18, 24 h	NaHS	IL-8; MAPK/ERK signaling phosphorylation	Reduced basal and IL-17/IL-22-induced IL-8 expression and secretion; Reduced MAPK/ERK phosphorylation. Reduction of inflammation events typical of psoriatic lesions
Chiarini et al. 2006	Int J Mol Med	Psoriasis	Human primary epidermal keratinocytes	25%, 50%, or 100% of Comano water dissolved in the culture medium, for 11 days	Comano spa's water (Trentino, Italy), rich in sodium, calcium and bicarbonate	VEGF-A	Reduced VEGF-A expression and secretion; Reduction of VEGF-A-mediated angiogenic, vessel permeabilizing, and chemotactic effects
Chiarini et al. 2006	Int J Mol Med	Psoriasis	Human primary epidermal keratinocytes	25%, 50%, or 100% of Comano water dissolved in the culture medium, from 3 to 15 days	Comano spa's water (Trentino, Italy), rich in sodium, calcium and bicarbonate	IL-6, CK-16, VEGF-A	Reduced IL-6, VEGF, and CK-16 release and expression. Reduction of inflammation, and neo-angiogenic phenomena of local psoriatic manifestations
Dal Pra et al. 2007	Int J Mol Med	Psoriasis	Human primary epidermal keratinocytes	25%, 50%, or 100% of Comano water dissolved in the culture medium, from 11 to 13 days	Comano spa's water (Trentino, Italy), rich in sodium, calcium and bicarbonate	IL-8, TNF-α	Reduced IL-8 and TNF-α intracellular levels and secretion rates. Reduction of inflammation events typical of psoriatic lesions
Karagülle et al. 2018	Int J Biometeorol	Psoriasis and rosacea	Human keratinocyte cell lines	10% of Bursa and Bolu thermal mineral waters dissolved in the culture medium, for 3 days		IL-1α, TNF-α, and VEGF	Reduced IL-1α, TNF-α, and VEGF gene expression; Reduction of inflammation, and neo-angiogenic phenomena of skin disease manifestations

Lee et al. 2012	Ann Dermatol	Skin disease	Human keratinocyte cell lines	50% of Yong-gung oncheon thermal spring water dissolved in the culture medium, for 1, 4, 10, and 24 h + LPS (10 $\mu$ L/mL)	HaCaT Thermal spring water (Yong-gung oncheon, Ganghwa-gun, Korea), rich in sulfur, magnesium, calcium and selenium	IL-6, IL-8; CD4+ T cells differentiation	Reduced IL-6 and IL-8 gene and protein expression; Attenuated differentiation of CD4+ T cells in Th1, Th2 and Th17. Attenuation of immune skin reactions
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## In vitro studies on human samples evaluating the beneficial effects of BT on immune response in musculoskeletal diseases

Immunological effects of thermal water in musculoskeletal diseases have been most widely studied in rheumatic disorders.

[Fox et al. in 2012](#) studied the ability of human primary chondrocytes and mesenchymal progenitor cells to synthesize H<sub>2</sub>S in response to pro-inflammatory mediators stimulation (IL-1 $\beta$ , IL-6, TNF- $\alpha$ , and lipopolysaccharide (LPS)), and their response to the exogenous slow-releasing H<sub>2</sub>S source (GYY4137). Endogenous H<sub>2</sub>S produced by the cells and the treatment with different concentrations of GYY4137 (50–500 mol/L for 18 h) significantly reduced cell death and oxidant-induced mitochondrial dysfunction, caused by inflammatory cytokines, via protein kinase B (Akt)/phosphoinositide 3-kinase (PI3K)-dependent signaling.

[Li et al. \(2013\)](#) assessed the effect of GYY4137 on LPS-caused release of inflammatory mediators from human arthritis synoviocytes and articular chondrocytes. After 18 h of treatment, GYY4137 demonstrated a prominent anti-inflammatory effect decreasing the production of nitrite (NO<sub>2</sub><sup>-</sup>), prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), TNF- $\alpha$ , and IL-6 from both cell types, reducing the levels and catalytic activity of inducible nitric oxide synthase (iNOS) and COX-2, and NF- $\kappa$ B activation induced by LPS. Similar results were obtained by [Burguera et al. in 2014](#) in a study on human OA chondrocytes stimulated with IL-1 $\beta$ , a pro-inflammatory cytokine, to reproduce the “OA-like effect”. The results of the research proved the ability of NaHS and GYY4137 to limit nitric oxide (NO), PGE<sub>2</sub>, and IL-6 released by the cells and at protein level, as well as the gene expression of NOS2, COX-2, prostaglandin E synthase (PTGES), IL-6, and NF- $\kappa$ B nuclear translocation activated by IL-1 $\beta$ . Furthermore, it was firstly demonstrated the anti-catabolic activity of these compounds through the downregulation of metalloproteinase (MMP-13).

[Ha et al. in 2015](#) studying human OA chondrocyte cultures treated with NaHS in the presence or not of IL-1 $\beta$  demonstrated that this compound markedly reversed the effects of IL-1 $\beta$  on the gene expression of COX-2, MMP-13, and NOS and on their production in the supernatant. In addition, NaHS inhibited the activation of the ERK/I $\kappa$ B $\alpha$ /NF- $\kappa$ B pathway induced by IL-1 $\beta$ . In the same year, [Sieghart et al. \(2015\)](#) investigated the effects of NaHS in OA fibroblast-like synoviocytes stimulated with IL-1 $\beta$ . The NaHS treatment reduced spontaneous and IL-1 $\beta$ -induced secretion of IL-6, IL-8, and RANTES, the expression of MMP-2 and MMP-14, and the phosphorylation of several MAPK proteins. On the contrary, sulfide source increased the phosphorylation of pro-survival factor Akt1/2, suggesting the ability of H<sub>2</sub>S to partially antagonize IL-1 $\beta$  stimulation through an action on the MAPK and the PI3K/Akt pathways.

Later, a similar research was conducted on OA cartilage extracts co-cultured with IL-1 $\beta$  and NaHS or GYY4137 for 21 days ([Vela-Anero et al. 2017](#)). At the end of the treatment, there was a reduction of the catabolic processes and a stimulation of the cell anabolism. Indeed, a decrease in glycosaminoglycan



destruction and MMP-3 and MMP-13 production caused by IL-1 $\beta$ , in addition to an increased synthesis of collagen type II alpha 1 chain and aggrecans, was observed in NaHS or GYY4137-treated cells.

In 2013, [Fioravanti et al.](#) studied the potential beneficial effect of Vetriolo thermal water (Trentino Alto Adige, Italy), a highly mineralized water, strongly acidic sulfate (SO<sub>4</sub><sup>-</sup>), rich in calcium, magnesium, and iron, in human OA chondrocytes cultivated in the presence of IL-1 $\beta$ . Thermal water was tested at different concentrations (100%, 50%, 25%), dissolved in the culture medium. After the treatment, there was a significant survival recovery rate, a reduction in NO levels, and in the expression of iNOS, as well as an enhancement of morphological characteristics of the cells, altered by IL-1 $\beta$ , in chondrocytes treated with 50% and 25% Vetriolo thermal water.

[Kloesch et al. in 2010](#) evaluated the effect of NaHS on fibroblast-like synoviocytes derived from rheumatoid arthritis (RA) patients. They demonstrated that IL-1 $\beta$ -induced expression of IL-6 was transiently and partially down-regulated with low concentrations of NaHS. H<sub>2</sub>S deactivates p44/42 MAPK (ERK1/2). Long-term exposure to H<sub>2</sub>S and high concentration of NaHS provided stimulatory effects, leading to reinforced activation of p38 MAPK and ERK1/2 accompanied by upregulation of IL-6 expression.

In [2012 Kloesch et al.](#) treated fibroblast-like synoviocytes derived from RA and OA-patients with NaHS. Low NaHS concentrations reduced IL-6 expression and activation of MAPK/ERK signalling, while high concentrations of H<sub>2</sub>S elevated the expression of pro-inflammatory genes in RA- and OA-fibroblast-like synoviocytes. This was accompanied by the activation of p38 and ERK1/2 MAPK. Thus, NaHS can upregulate the expression of a series of pro-inflammatory genes in RA and OA-FLS.

In [2012 Kloesch et al.](#) on a human chondrocyte cell line treated with NaHS dissolved in the culture medium, for 15, 30, 45 and 60 min, demonstrated that IL-6 and IL-8 expression and the activation of p38/MAPK/ERK and NF- $\kappa$ B signaling were reduced.

On bone-derived cells, only a limited number of in vitro studies were performed to investigate the properties of exogenous sources of H<sub>2</sub>S. In a research performed on human differentiated osteoclasts ([Gambari et al. 2014](#)) after an incubation period ranging from 72 h to 6 days in presence of NaHS, a significant dose-dependent decrease in osteoclast differentiation and intracellular ROS levels, and an upregulation of nuclear factor erythroid 2-related factor 2 (NRF2) activity, related to an increased transcription of the downstream antioxidant genes, were observed.

All the results of these studies are reported in Table II.

Authors	Journal	Pathology	Experimental model	Treatment	Mineral water or inorganic or organic components	Biochemical parameters	Results
Fox et al. 2012	J Cell Mol Med	Rheumatoid Arthritis	Human primary articular chondrocytes	IL-1 $\beta$ , IL-6 and TNF- $\alpha$ (5 ng/mL) for 6, 12 and 18 h + GYY4137 (50–500 mol/L) dissolved in the culture medium, for 12 h	GYY4137	Cell death; Mitochondrial membrane potential	Reduced cell death and oxidant-induced mitochondrial dysfunction Limitation of inflammation in chronic inflammatory diseases

Li et al. 2013	J Cell Mol Med	Rheumatoid arthritis	Human primary arthritis synoviocytes and chondrocytes	(0.1–0.5 mM) dissolved in the culture medium, for 18 h + LPS (10 µg/mL)	GY4137		Reduced IL-6, TNF-α, PGE2 and NO production, COX-2 and iNOS catalytic activity, and NF-κB activation Reduction of inflammatory processes of arthritis
Burguera et al. 2014	Osteoarthr Cartil	Osteoarthritis	Human primary OA chondrocytes	NaHS and GY4137 (0.05–1 mM) dissolved in the culture medium, for 24 or 48 h + IL-1β (5 ng/mL)	NaHS and GY4137	IL-6, PGE2, PTGES, COX-2; NO, NOS2; MMP-13; NF-κB signaling activation	Reduced IL-6, PGE2, and NO release and protein level, IL-6, PTGES, COX-2, and NOS2 gene expression, and NF-κB nuclear translocation Reduction of inflammatory and degrading processes of OA damage
Ha et al. 2015	Int J Mol Med	Osteoarthritis	Human primary OA chondrocytes	NaHS (0.06–1.5 mM) dissolved in the culture medium, for 24 h + IL-1β (10 ng/mL)	NaHS	COX-2, iNOS, MMP-13; ERK/IκBα/NF-κB signaling activation	Reduced COX-2, iNOS, MMP-13 release and gene expression; Inhibited ERK/IκBα/NF-κB activation Reduction of degrading processes of OA damage
Sieghart et al. 2015	J Cell Mol Med		Human primary OA fibroblast-like synoviocytes	NaHS (0.06–1 mmol/L) dissolved in the culture medium, for 1 h + IL-1β (10 ng/mL)	NaHS	IL-6, IL-8; MMP-2, MMP-14; MAPK and Akt1/2/PI3K protein phosphorylation	Reduced IL-6 and IL-8 secretion, MMP-2 and MMP-14 gene expression, and MAPK phosphorylation; Increased Akt1/2 phosphorylation Reduction of inflammatory and degrading processes of OA damage
Vela-Anero et al. 2017	Nitric Oxide	Osteoarthritis	Human OA cartilage disks	NaHS or GY4137 (200 or 1000 µM) dissolved in the culture medium, for 21 days + IL-1β (5 ng/mL)	NaHS and GY4137	MMP-3, MMP-13; Col2a1, glycosaminoglycans, aggrecans	Reduced MMP-3 and MMP-13 production, and increased Col2a1, glycosaminoglycans, and aggrecans synthesis Reduction of degrading processes of OA damage

Fioravanti et al. 2013	J Biol Regul Homeost Agents	Osteoarthritis	Human primary OA chondrocytes	25%, 50%, or 100% of Vetricolo thermal water dissolved in the culture medium, for 48 h+ IL-1 $\beta$ (5 ng/mL)	Vetricolo thermal water (Trentino Alto Adige Italy), strongly acidic sulfate, rich in calcium, magnesium and iron	NO, iNOS; Cell viability and apoptosis; Morphological assessment	25%, 50% of Vetricolo water increased survival recovery rate, reduced NO levels, iNOS expressions, and apoptosis Enhanced morphological characteristics Reduction of degrading processes of OA damage
Kloesch et al. 2010	Cell Biol Int	rheumatoid arthritis	fibroblast-like synoviocytes	NaHS (200 mM)	NaHS	IL-6; activation/deactivation of MAPKs; p38 and p44/42 MAPK (ERK1/2)	IL-1beta-induced expression of IL-6 transiently and partially down-regulated with low concentrations of NaHS. H2S deactivates p44/42 MAPK (ERK1/2). Long-term exposure to H2S provides stimulatory effects, leading to reinforced activation of p38 MAPK and ERK1/2 accompanied by upregulation of IL-6 expression.
Kloesch et al. 2012	Immunol Lett	Rheumatoid Arthritis and Osteoarthritis	RA and OA human fibroblast-like synoviocytes	NaHS (1.0 mM) dissolved in the culture medium, for 1, 3, 6, 12 h	NaHS	IL-6, IL-8, COX-2; MMP-2, MMP-3, MMP-14; P38/MAPK/ERK protein expression	Increased IL-6, IL-8, COX-2 and p38/MAPK/ERK expression
Kloesch et al. 2012	Rheumatol Int	Rheumatoid Arthritis	Human chondrocyte cell line	NaHS (0.125 and 1.0 mM) dissolved in the culture medium, for 15, 30, 45 and 60 min + MAPK inhibitors (1 and 5 $\mu$ M) + IL-1 $\beta$ (5 ng/mL) for 1 h	NaHS	IL-6, IL-8; P38/MAPK/ERK and NF-kB signaling activation/-deactivation	Reduced IL-6 and IL-8 expression and activation of p38/MAPK/ERK and NF-kB signaling Reduction of inflammatory processes of arthritis

Gambari et al. 2014	Pharmacol Res	Osteoporosis	Human differentiated osteoclasts	NaHS (50–300 µM) dissolved in the culture medium, for 72 h to 6 days	NaHS	Osteoclasts differentiation; ROS production, NRF2, KEAP1, NQO1, and PRDX1	Decreased osteoclast differentiation, intracellular ROS levels; Upregulated NRF2 protein expression and nuclear translocation, and increased antioxidant gene
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### In vitro studies on animal samples evaluating the beneficial effects of BT on immune response in musculoskeletal diseases

[Xu et al. \(2011\)](#) showed the proliferative, antioxidant, and anti-inflammatory effects of 4 h of treatment with NaHS in hydrogen peroxide-stimulated murine osteoblast-like cell line. After the treatment, an increased cell viability, cell proliferation (by enhancing alkaline phosphatase activity), and reduced apoptosis, caused by H<sub>2</sub>O<sub>2</sub>, were observed. Furthermore, the H<sub>2</sub>S source increased superoxide dismutase activity, while it decreased reactive oxygen species production, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity, and NO and TNF-α release, probably through p38 and ERK1/2 MAPKs pathways. These results were confirmed by [Lv et al. \(2017\)](#), in an analogous experimental study, examining the effect of GYY4137 added at the culture medium for 4 h in the presence of H<sub>2</sub>O<sub>2</sub>. They confirmed the proliferative and antioxidant effects of NaHS.

Authors	Journal	Pathology	Experimental model	Treatment	Mineral water or inorganic or organic components	Biochemical parameters	Results
Xu et al. 2011		Osteoporosis	Murine osteoblast-like cell line (MC3T3-E1)	NaHS (100 µM) dissolved in the culture medium, for 4 h + (H <sub>2</sub> O <sub>2</sub> ) (400 µM)	NaHS	Viability, proliferation and apoptosis; NO, ALP, SOD, NADPH oxidase p38/ERK1/2/MAPKs activation	Increased viability, cell proliferation, ALP and SOD activities; Decreased apoptosis, NO release and NADPH oxidase activity, and p38/ERK1/2/MAPKs activation Proliferative and antioxidant effects against osteoporosis damage
Lv et al. 2017	Am J Transl Res	Osteoporosis	Murine osteoblast-like cell line (MC3T3-	GYY4137 (100 µM) dissolved in the culture	GYY4137	Viability, proliferation, Runx2, and apoptosis; NO, ALP, and SOD, ERK1/2 activation	Increased viability, cell proliferation

			E1)	medium, for 4 h + (H <sub>2</sub> O <sub>2</sub> ) (400 μM)			n, ALP and SOD activities, and Runx2 gene expression; Decreased apoptosis, NO release, and ERK1/2 activation Proliferative and antioxidant effects
Liu et al. 2017	Biochimie	Osteoporosis	Rat primary osteoblasts	NaHS (400 μmol/L) dissolved in the culture medium, for 12 h	NaHS	Osteoblast proliferation and mineralization; Apoptosis; KATP protein expression	Decreased cell proliferation, and increased the number of apoptotic cells, osteoblast mineralization, and KATP protein expression Reduction of osteoporosis damage

In 2017, [Liu et al.](#) pretreated rat primary osteoblast cultures with 400 μmol/L NaHS for 30 min, followed by an incubation with high glucose concentration, for 12 h, before the analysis of cell proliferation, apoptosis, and mineralization. NaHS significantly prevented osteoblast injury induced by glucose, through decreasing the rate of cell proliferation, increasing the number of apoptotic cells, and blocking the high glucose-induced osteoblast mineralization inhibition, via activating ATP-sensitive potassium (KATP) channels.

All the results of these studies are reported in Table III.

### In vitro studies on human samples evaluating the beneficial effects of BT on immune response in inflammatory diseases

The protective effects of H<sub>2</sub>S and of its exogenous sources on cellular immune response was firstly investigated by [Rinaldi et al. \(2006\)](#) in a study on purified human neutrophils, eosinophils, and lymphocytes of human donors treated with NaHS at different concentrations for 24 h. The Authors found an increased short-term survival of neutrophils delaying the onset of apoptosis, while no changes in lymphocytes or eosinophils were observed. The pro-survival effect of NaHS was due to its inhibitory activity on caspase-3 cleavage and p38/MAPK phosphorylation at the protein level.



A similar experimental protocol was performed, 1 year later, by [Mirandola et al. \(2007\)](#) in peripheral blood lymphocytes purified from healthy subjects. The cells were incubated with different concentrations of NaHS for 24 h to examine its role in regulating cell death and cytotoxicity, and its anti-inflammatory properties. At the end of the treatment, a dramatically decreased proliferation of surviving lymphocyte subsets, CD8+ T and NK cells, as well as a reduced IL-2 production, induced in response to mitogens, were observed.

Also, [Sulen et al. \(2016\)](#) investigated the ability of H<sub>2</sub>S sources to regulate the activation of signalling transduction pathways implicated in immune response. Human peripheral blood mononuclear cells (PBMCs) isolated from healthy donors were stimulated with NaHS at concentrations of 10, 100, or 1000  $\mu$ M for 10 min, and the phosphorylation of p38/MAPK, NF- $\kappa$ B p65, AKT, and cAMP response element-binding protein (CREB) was analysed. NaHS induced phosphorylation of p38, AKT, and CREB, but not NF- $\kappa$ B. The NaHS-induced signal transduction pathway in human primary immune cells may have relevance for the role of sulfides in inflammation.

The proliferative activity of H<sub>2</sub>S donors was also demonstrated in experiments carried out on peripheral blood lymphocytes isolated from patients with systemic lupus erythematosus ([Han et al. 2013](#)). Various concentrations of NaHS and GYY4137 were added to the culture medium of the cells for different time in order to evaluate the cell viability, cell cycle distribution, and expression of proteins involved in pathological pathways regulating autoimmune response. H<sub>2</sub>S donors inhibited the abnormal activation and proliferation of lupus lymphocytes through the AKT/GSK3 $\beta$  pathway.

Another in vitro study has been performed in activated human neutrophils isolated from blood of healthy donors and treated with increasing amounts of the sulfurous thermal water of Acqui Terme (Piemonte, Italy) for 15 min ([Braga et al. 2008](#)). The cells were stimulated with N-formyl-methionyl-leucyl-phenylalanine and phorbol-12-myristate-13-acetate before and after incubation with sulfurous water, then the luminol-amplified chemiluminescence methodology was used to investigate ROS and reactive nitrogen species (RNS) release. The results showed that this mineral water significantly reduced the luminol-amplified chemiluminescence induced by the stimulus.

The same Authors performed a similar experiment treating neutrophils for 15 min with different concentrations of the above mentioned sulfurous water or NaHS ([Braga et al. 2010](#)); elastase release was evaluated by spectrofluorimetry, and elastolytic activity of the cells was determined by measuring the diameter of the area of elastinolysis on elastine-agarose gel plates. Sulfurous water and NaHS significantly inhibited elastase release but did not show any direct elastolytic activity. This finding revealed the possible contribution of sulfurous water in controlling the inflammatory processes of upper and lower airway diseases.

In [2013](#), [Prandelli et al.](#) used human primary monocytes to test the beneficial effects of Sirmione thermal water (Lombardia, Italy), very rich in sodium chloride, bromide, and iodide, and of NaHS at concentration of 2.5 mM. Thermal water or NaHS was added to the culture medium of the cells for 24 h in the presence or not of 100 ng/mL of LPS, then the release of pro-inflammatory cytokines and the formation of ROS were evaluated. NaHS efficiently blocked the production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, CXCL8, and CCL5 induced by LPS, and limited ROS formation and antioxidant enzymes activity. Sirmione water did not induced the same results, but only enhanced the release of IL-10, probably due to the low concentration of S-based compounds. The Authors attested the anti-inflammatory and antioxidant properties of S-based compounds against the main manifestation of chronic inflammatory and age-related illness.

Given the important role of Th17/Treg cell ratio in the onset and clinical evolution of immune-mediated pathologies, [Vitale in 2018](#) investigated the effects of exogenous H<sub>2</sub>S on human resting CD4 T cell polarization to Th17 and/or Treg phenotype. NaHS treatment increased Foxp3 mRNA levels in CD4+ T

cells cultured under Treg-polarizing conditions. However, NaHS was also able to increase ROR $\gamma$ T mRNA levels in CD4+ T cells under Th17 polarizing conditions, suggesting a role of sulfurs in the activation of both polarization pathways.

All the results of these studies are reported in Table IV.

Authors	Journal	Treatment	Experimental model	Mineral water or inorganic or organic components	Pathology	Biochemical parameters	Results
Rinaldi et al. 2006	Lab Investig	NaHS (from 0.23 to 3.66 mM) dissolved in the culture medium, for 24 h  + p38/MAPK inhibitors (30–60 $\mu$ M)	Human purified neutrophils, eosinophils or lymphocytes	NaHS	Inflammatory processes of respiratory tract	Cell viability and apoptosis; P38/MAPK signaling activation/deactivation	Short-term survival of neutrophils delaying the onset of apoptosis  Reduced caspase-3 cleavage and p38/MAPK phosphorylation in neutrophils  Accelerate the resolution of inflammatory processes
Mirandola et al. 2007	J Cell Physiol	NaHS (from 0.20 to 4.0 mM) dissolved in the culture medium, for 24 h  + caspase inhibitors (30 $\mu$ M)	Human purified peripheral blood lymphocytes	NaHS	Inflammatory processes	Cell viability and apoptosis; IL-2	Decreased proliferation of lymphocyte subsets, CD8+ T and NK cells, and reduced IL-2 production  Accelerate the resolution of inflammatory processes

Sulen et al. 2016	Pharmacol Res	NaHS (10, 100 or 1000 µM) dissolved in the culture medium, for 10 min	Human peripheral blood mononuclear cells (PBMCs)	NaHS	Inflammatory processes	p38/MAPK, NF-κB p65, AKT and CREB phosphorylation	Induced p38/MAPK, AKT, and CREB phosphorylation  Reduction of inflammatory processes
Han et al. 2013	Cell Physiol Biochem	NaHS (0.25, 0.5, 1, 2, 4 and 8 mM) and GYY4137 (200, 400, 800, 1600 µM) dissolved in the culture medium, for 0.5, 1, 2, 4, 6, 12, 24, 36, 48 h	Human purified peripheral blood lymphocytes	NaHS and GYY4137	Inflammatory processes of systemic lupus erythematosus	Cell viability, cell cycle distribution; AKT (ser473), GSK3β (ser9), p27Kip1 and p21CIP1	Increased cell proliferation and S phase distribution of cell cycle;  Decreased AKT (ser473), GSK (ser9), and increased p27Kip1 and p21CIP1 expression and phosphorylation  Reduction of inflammatory processes of systemic lupus erythematosus
Braga et al. 2008	Respiration	Sulfurous thermal water (different concentrations) dissolved in the culture medium, for 15 min  + N-formyl-methionyl-leucyl-phenylalanine/phorbol-12-myristate-13-acetate	Human purified neutrophils	Sulfurous thermal water (Acqui Terme, Piemonte, Italy), which contains different HS groups concentrations	Inflammatory processes	ROS and RNS	Reduced ROS and RNS release at 0.94 to 15.5 µg/mL of HS  Reduction of inflammatory processes
Braga et al. 2010	Ther Adv Respir Dis	Sulfurous water or NaHS (from 4.5 to 18 mg/mL) dissolved in the culture medium, for 15 min	Human purified neutrophils	Sulfurous thermal water (Acqui Terme, Piemonte, Italy) and NaHS	Inflammatory processes of upper and lower airway diseases	Elastase release; elastolytic activity	Inhibited elastase release  Reduction of inflammatory processes

Prandelli et al. 2013	Int J Immunopathol Pharmacol	Sirmione thermal water or NaHS (2.5 mM) dissolved in the culture medium, for 24 h + LPS (100 ng/mL)	Human primary monocytes	Sirmione thermal water (Lombardia, Italy), rich in sodium chloride, bromine and iodine	Chronic inflammatory and age-related illness	TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12; CXCL8, CCL5; ROS, antioxidant enzymes	NaHS reduced TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-12, CXCL8, CCL5 production, ROS formation and antioxidant enzymes;  Sirmione water enhanced IL-10 release  Reduction of chronic inflammatory and age-related illness manifestations
Vitale 2018	Bol Soc Esp Hidrol Méd	exogenous H2S on human resting CD4 T cell polarization to Th17 and/or Treg phenotype	differentiated ex-vivo human resting CD4+ (Th0) T cells to Th17 or Treg lineages	NaHS	immune-mediated pathologies	CD4 T cell polarization to Th17 and/or Treg phenotype	increased Foxp3 mRNA levels in CD4+ T cells cultured under Treg-polarizing conditions  increased ROR $\gamma$ T mRNA levels in CD4+ T cells under Th17 polarizing conditions

### In vitro studies on animal samples evaluating the beneficial effects of BT on immune response in inflammatory diseases

H2S donors were used to examine their role in mediating the immune response in inflammatory bowel diseases on animal samples: nanomolar levels of Na2S and GYY4137 were employed to treat primary mouse T lymphocytes (CD3+) and OT-II CD4+ T cells to establish whether endogenous H2S production is required for T cell activation, in mediating inflammatory response in such kind of diseases ([Miller et al. 2012](#)). H2S donors enhanced T cell activation assessed by CD69 expression, IL-2 expression, and CD25 levels, with a maximum capacity at 300 nM. Besides, activation increased the capacity of T cells to synthesize endogenous amounts of H2S via increased expression of cystathionine  $\gamma$ -lyase and cystathionine  $\beta$ -synthase. These findings lead to define H2S as an endogenous and exogenous immunomodulatory molecule in T cells signal.

These results are reported in Table V.

Authors	Journal	Treatment	Experimental model	Mineral water or inorganic or organic components	Pathology	Biochemical parameters	Results
Miller et al. 2012	J Biol Chem	H <sub>2</sub> S (50–500 nM) dissolved in the culture medium, for 4, 10, and 24 h	Primary mouse T lymphocytes (CD3+), OT-II CD4+ T cells	H <sub>2</sub> S	Inflammatory processes of bowel diseases	CD69, CD25; IL-2; cystathionine $\gamma$ -lyase, cystathionine $\beta$ -synthase	Enhanced T cell activation, CD69, and IL-2 expression, and CD25 levels;  Increased cystathionine $\gamma$ -lyase and cystathionine $\beta$ -synthase expression  Reduction of inflammatory processes of bowel diseases

## RCT and clinical trials evaluating the beneficial effects of BT on immune response

Most of the clinical studies and RCT evaluating the effects of thermal waters on the immune response focused on musculoskeletal diseases.

[Bellometti et al. in 1997](#) conducted a study enrolling a group of 22 osteoarthrosic patients, 12 males and 10 females, aged  $63.6 \pm 6.6$  years, diagnosed of OA on the basis of their clinical symptoms and X-ray examination. The subjects underwent a total of 12 mud pack treatments, with daily frequency, in a thermal resort (Abano-Montegrotto Terme, Veneto, Italy). Mature thermal mud was applied to the whole body for 20 min at 40°C, followed by a bath for 10–12 min at 37–38°C. Blood samples were collected 30 min before the first mud pack and 2 h after the last one. The results documented specific increases of insulin growth factor 1 (IGF-1) and decreases of TNF- $\alpha$  in serum of OA patients after 12 days of mud pack application. The values of serum IGF-1 and TNF- $\alpha$  of a control group of 22 patients matching the mud pack-treated group and receiving only the hot bath did not show any change.

In [1998 Bellometti et al.](#) investigated whether mud pack therapy exerts a protective effect on the cartilage and is able to induce pain relief by reducing the inflammatory reaction. They enrolled 31 subjects undergoing mud pack therapy and collected blood samples before and after the therapy to assay serum levels of prostaglandin (PGE<sub>2</sub>) and leukotriene (LTB<sub>4</sub>), compounds with potent inflammatory and algescic properties. The study showed a decrease in PGE<sub>2</sub> and LTB<sub>4</sub> serum levels in all the samples after mud pack therapy.

In [2016 Pascarelli et al.](#) conducted a study aiming to evaluate the effects of mud-bath therapy on serum biomarkers in patients with knee OA. The study group comprised 103 patients with primary symptomatic bilateral knee OA who were randomly assigned to receive a cycle of mud-bath therapy over a period of 2 weeks or to continue their standard therapy alone. Clinical and biochemical parameters were assessed



at baseline and after 2 weeks. Clinical assessments included global pain score on a visual analogue scale (VAS) and the Western Ontario and McMaster Universities Index (WOMAC) subscores for knee OA. Cartilage oligomeric matrix protein (COMP), C-terminal cross-linked telopeptide type II collagen (CTX-II), myeloperoxidase (MPO) and high sensitivity C-reactive protein (hsCRP) serum levels were assessed by ELISA. At the end of the mud-bath therapy a statistically significant improvement in VAS and WOMAC subscores was observed. The evaluation of serum biomarkers showed a significant increase of CTX-II only, perhaps due to an increase of cartilage turnover induced by thermal stress. No significant modification was found in hsCRP serum levels.

[Ortega et al. in 2017](#) conducted an investigation having the aim to evaluate whether an anti-inflammatory effect together with an improvement of the regulation of the interaction between the inflammatory and stress responses underlies the clinical benefits of pelotherapy in OA patients. This study evaluated the effects of a 10-day cycle of pelotherapy at the spa centre 'El Raposo' (Spain) in a group of 21 OA patients diagnosed with primary knee OA. In addition to clinical assessments including pain perception, quality of life, and physical function evaluation, serum inflammatory cytokine levels (IL-1 $\beta$ , TNF- $\alpha$ , IL-8, IL-6, IL-10 and TGF- $\beta$ ) were evaluated. Circulating neuroendocrine-stress biomarkers, such as cortisol and extracellular 72 kDa heat shock protein (eHsp72), were measured. After the cycle of mud therapy, OA patients improved pain perception, stiffness and physical function, and they reported a better health-related quality of life. Serum concentrations of IL-1 $\beta$ , TNF- $\alpha$ , IL-8, IL-6 and TGF- $\beta$ , as well as eHsp72, were markedly decreased. Besides, systemic levels of cortisol increased significantly.

[Galv ez et al. in 2018](#) investigated the role for regulatory T cells in the anti-inflammatory effects of balneotherapy with mud applications (mud therapy) commonly used for the treatment and rehabilitation of elderly patients with OA. The second objective of their investigation was to assess whether the neutrophil-mediated innate response is affected by these anti-inflammatory effects. They enrolled thirty-six elderly patients with knee osteoarthritis who underwent a 10-day cycle of balneotherapy. They received daily sessions of whole-body mud therapy at 40–42 °C, using mineral medicinal water and mud. IL-8 and TGF- $\beta$  serum concentrations, percentage of circulating CD4+ CD25+ FOXP3+ and CD8+ CD28– regulatory T cells, and neutrophil phagocytic capacity were evaluated at baseline and at the end of the intervention. Clinical assessments including knee flexion and extension angle, pain, stiffness, physical function and health-related quality of life significantly improved after the treatment. Circulating concentrations of IL-8 and TGF- $\beta$  decreased, which correlated with decreased pain perception and improved knee flexion, respectively. Percentage of CD4+ regulatory T cells decreased, whereas CD8+ regulatory T cells increased. Neutrophil functional capacity increased.

[Tarnier et al. in 2009](#) analyzed the effect of mild whole-body hyperthermia, which is a widely used balneotherapy modality for clinically inactive ankylosing spondylitis (AS) in rehabilitative medicine, on the systemic levels of proinflammatory cytokines. 12 male patients with AS and 12 healthy control subjects received nine cycles of whole-body hyperthermia. Serum samples were taken at the beginning of the last cycle and at 1, 6, and 24 h for measurement of TNF- $\alpha$ , IL-1 $\beta$  and IL-6. Significant differences in cytokines levels were found between both groups. In AS patients, hyperthermia caused a significant reduction of all cytokines by 40-50%.

[Shehata et al. in 2006](#) conducted a study to find evidence for a biological response to speleotherapy in patients with AS, for which reduction of joint pain has been reported after spa treatment, and to study the involvement of the anti-inflammatory cytokine TGF-beta1 in this response. 83 patients with AS were treated in Badgastein (Austria) for 3-4 weeks. Therapy included active exercises, hyperthermia and exposure to low doses of radon in a former mine. Response to therapy was assessed from measurement of morning pain and immunoassay of serum levels of TGF-beta1 before and after therapy. 10 AS patients who received conventional therapy and 10 patients with low back pain (LBP) served as controls. After spa therapy, a significant increase in TGF- $\beta$ 1 (total and active) was found in AS patients. There was a

moderate increase in active TGF- $\beta$ 1 in patients with LBP and no significant change was observed in the patients treated with conventional therapy. So, these results demonstrate a significant increase in circulating TGF- $\beta$ 1 in patients with AS after the combined spa-exercise therapy. The results also provide evidence for a biological response to speleotherapy and suggest that TGF- $\beta$ , through its antiinflammatory function, may play a role in this response.

[Ardic et al. in 2007](#) carried out a study to investigate the clinical effects of balneotherapy in the treatment of Fibromyalgia Syndrome (FMS) and to determine if balneotherapy influences serum levels of inflammation markers, IL-1, PGE2 and LTB4. 24 primary fibromyalgia female patients aged between 33 and 55 years were included in the study. The patients were randomly assigned in two groups: the first group received 20-min bathing, once in a day for five days per week for 3 weeks (total of 15 sessions), the second group did not receive balneotherapy. Ten healthy women were recruited as the control group. FMS patients were evaluated by tenderness measurements (tender point count and algometry), Visual Analogue Scale, Beck's Depression Index, Fibromyalgia Impact Questionnaire. Serum PGE2, LTB4 and IL1- $\alpha$  levels were measured in all three groups. The biochemical measurements and clinical assessments were performed before and at the end of the treatment. Statistically significant alterations in pain perception, numbers of tender points, Fibromyalgia Impact Questionnaire score, Beck's Depression Index and PGE2 levels, were found after the balneotherapy between the first and the second group. Mean PGE2 level of FMS patients were higher compared to healthy control group and decreased after the treatment period only in the group who received balneotherapy. As in the other groups detectable IL-1 and LTB4 measurements were insufficient, statistical analysis was performed only in the first group. In this group after balneotherapy IL-1 and LTB4 significantly decreased.

In [2013 Eysteinsdóttir et al.](#) investigated the effects of bathing in geothermal seawater in addition to the NB-UVB therapy in patients suffering from psoriasis. Six patients with psoriasis were treated with bathing in geothermal seawater two times daily combined with NB-UVB five times/week for 2 weeks and six patients were treated with NB-UVB therapy only, three times/week for 8 weeks. Disease severity (Psoriasis Area and Severity Index, PASI), chemokines, inflammatory cytokines, T cells and Toll-like receptors in the blood and skin samples were evaluated on enrolment and at 1, 3, and 8 weeks. Compared with healthy controls, psoriasis patients with active disease had significantly higher proportion of peripheral CLA+ T cells expressing CCR10 and CD103 and T cells with both Th1/Tc1 (CD4+/CD8+ IFN- $\gamma$ + or TNF- $\alpha$ + cells) and Th17/Tc17 (CD4+CD45R0+IL-23R+, CD4+/CD8+ IL-17A+ or IL-22+ cells) phenotypes. Both treatments gave a significant clinical effect; however, bathing in geothermal seawater combined with NB-UVB therapy was more effective than NB-UVB therapy alone. This clinical improvement was reflected by a reduction in circulating CLA+ peripheral blood T cells and by a decreased Th1/Th17 and Tc1/Tc17 inflammatory response. These findings suggest that the inflammatory response in psoriasis is predominantly driven by both CD4+ and CD8+ skin-homing tissue retaining T cells of the Th17/Tc17 lineages.

The radioactive and thermal effects of radon hot spring were biochemically compared under a sauna room or hot spring conditions with a similar chemical component ([Yamaoka et al. 2004](#)). The results showed that the radon and thermal therapy enhanced the antioxidation functions, such as the activities of superoxide dismutase (SOD) and catalase, which inhibit lipid peroxidation and total cholesterol produced in the body. Moreover the therapy enhanced concanavalin A (ConA)-induced mitogen response and increased the percentage of CD4 positive cells, which is the marker of helper T cells, and decreased the percentage of CD8 positive cells, which is the common marker of killer T cells and suppressor T cells, in the white blood cell differentiation antigen (CD8/CD4) assay. Furthermore, the therapy increased the levels of alpha atrial natriuretic polypeptide (alpha ANP), beta endorphin, adrenocorticotrophic hormone (ACTH), insulin and glucose-6-phosphate dehydrogenase (G-6-PDH), and it decreased the vasopression level. The results were on the whole larger in the radon group than in the

thermal group. The findings suggest that radon therapy contributes more to the prevention of life-style-related diseases related to peroxidation reactions and immune suppression than to thermal therapy.

All the results of these studies are reported in Table VI.

First Author	Journal	N	Pathology	Age	Intervention	Treatment	Comparison	Type of water/inorganic or organic component	Systemic Inflammatory Biomarkers	Stress biomarkers	Results
Bellomitti et al. 1997	Clinica Chimica Acta	2	OA	63.6 year	mud pack treatments	12 application of 'Mature' thermal mud to the whole body for 20 min at 40°C, followed by a bath for 10–12 min at 37–38°C	yes (22 control group only hot bath)	Nd	serum IGF 1 and TNFa		Differences between mean values found before and after the mud pack statistically significant
Bellomitti et al. 1998	J Investig. Med	3	health y		Mud pack Therapy				prostaglandin (PGE2) and leukotriene (LTB4)		decreased PGE2 and LTB4 serum with no correlation between the PGE2 and LTB4 decreases
Pascarelli et al. 2016	IMAJ	1	OA	68.4	mud bath therapy	2 weeks daily local mud-packs and baths	control group (n=50) regular care routine (exercise, symptomatic drugs, SYSAD OA, intra-articular hyaluronic	water of the Chiancia no Spa Resort (Siena, Italy)	Cartilage oligomeric matrix protein (COMP), C-terminal cross-linked telopeptide type II collagen (CTX-II), myeloperoxidase (MPO) and high sensitivity		No significant modification in serum levels of COMP, MPO and hsCRP in either group  Significant increase in CTX-II serum levels in the mud-bath group after the treatment

							acid).		C-reactive protein (hsCRP) serum		
Ortega et al. 2017	Int J Biometeorol	2 1	OA knee	62- 77	Whole body Peloidotherapy	1 session/ die x 10	no	SiO <sub>2</sub> , CaO, Al <sub>2</sub> O <sub>3</sub> and Fe <sub>2</sub> O <sub>3</sub>	IL-1b, TNFa, IL- 8, IL-6, TGF B	Serum Cortisol, eHsp72	Decreased serum concentrations of the pro-inflammatory cytokines IL-1β, TNF-α, IL-8, the regulatory cytokine IL-6 and the anti-inflammatory cytokine TGF-β.  Increased systemic levels of cortisol  significant decreased circulating levels of eHsp72
Galvéz et al. 2018	INTERNATIONAL JOURNAL OF HYPERTHERMIA	3 6	Knee OA	70	whole-body mud therapy at 40–42°C	10-day cycle	no	SiO <sub>2</sub> , CaO, Al <sub>2</sub> O <sub>3</sub> and Fe <sub>2</sub> O <sub>3</sub>	IL-8, TGFb, CD4, CD 25, FOXP3, CD28, CD8, Neutrophils		Decreased circulating concentrations of IL-8 and TGF-b  Reduced percentage of CD4 CD25 FOXP3 Treg cells  Increased CD8β CD28– Treg cell  Significantly increased percentage of phagocytic neutrophils and phagocytic activity of neutrophils
Tarner et al. 2009	Clinical Rheumatology	1 2	ankylosing spondylitis	mean age 33.4 years	whole-body hyperthermia full bath	nine cycles (initial water temperature of 36°C that was increased gradually by 1°C	yes (12 health y control )	nd	serum levels of the cytokines TNF-α, IL- 1B e IL-6		Decrease levels of TNF-α, IL-1β, and IL-6  No change detectable 1 h after baseline, but by 6 h after baseline, significant reduction. At

						every 5 min to 40°C. This temperature was maintained until the body temperature reached 38.5°C)				24 h after the treatment, mean TNF- $\alpha$ levels were reduced by 50%, mean IL-1 $\beta$ by 40%, and mean IL-6 by 43%. The continued decrease of cytokine levels between 6 and 24 h after baseline was also significant.
Shehata et al. 2006	The Middle European Journal of medicine	83	AS	30-73	spa exercises therapy	3-4 weeks spa-exercise therapy-in addition, outdoor exercises, physiotherapy, hydrotherapy and massage	yes (control group with 10 patients with LBP and same treatment, second control group with 10 patients AS and no treatment)	radon concentration up to 4.5 nCi/l; temperature 38-41°C; relative humidity 70-98%	tgf-b	Increased total and active TGF-B1 in the majority of AS patients
Ardic F. 2007	Rheumatol Int	12	Fibromyalgia Syndrome	mean age 43	Balneo therapy	Group 1 (n = 12) received bathing for 20 min a day, for 5 days per week for 3 week	yes (9 group no treatment+ 10 control group healthy women)	HCO <sub>3</sub> , SO <sub>4</sub>	erythrocyte sedimentation rate (ESR), serum C-reactive protein (CRP), rheumatoid factor (RF), Serum PGE <sub>2</sub> , LTB <sub>4</sub> and IL1-a (before and at the end of general period of	Reduction of the Increased serum PGE <sub>2</sub> level in FM subjects.  Decreased IL-1 and LTB <sub>4</sub>



									therapy)	
Eysteins dóttir et al. 2013	Scandina vian Journal of Immunol ogy	1 2	psorias is	(me an: 36.7 year s [ran ge: 19– 57]	bathing in geothe rmal seawat er	bathing in geother mal seawate r twice daily for at least 1 h combine d with NB-UVB therapy 5 days per week for 2 weeks	yes (Of the 12 patient s enrolle d, six receive d treatm ent and 6 were treated with NB- UVB therap y)	The temperat ure was 36°C, the radon concentr ation was 2,080 Bq/m3	chemokin es, inflamma tory cytokines, T cells and Toll- like receptors in the blood and skin samples were evaluated on enrolmen t and at 1, 3 and 8 weeks.	Marked reduction in circulating Th17 (IL- 23R+CD4+ T cells) after only one week of combined treatment  Reduction in the amount of IL-23R expressed by these cells and their IL- 17/IL-22 cytokine secretion.  Significant reduction in Tc17 T cells (producing IL- 17 and IL-22) after both of the treatments  Reduction in the Th1 and Tc1 phenotype (IFN-c and TNF-a production)
Yamaoka et al. 2004	J. Radiat. Res.	1 5	Health y	20- 40	a hot bathro om with a high concentr ation of radon	On days 1, 3, 5, 8, and 10, the inhalati on of vapor from each hot spring under a conditio n of high humidit y (about 90%).	yes (3 groups : radon, therma l, and control .)	The temperat ure was 36°C, the radon concentr ation was 2,080 Bq/m3	C4 positive cells and CD 8 positive cells	On day 5, increased percentage of CD4 positive cells compared to the level before the first treatment. On days 5 and 10, significantly increased percentage of CD4 positive cells and significantly decreased per centage of CD8 positive cells in the radon group

## Conclusion

In *in vitro* human samples, H<sub>2</sub>S donors (NaHS and GYY4137) were demonstrated to exert anti-inflammatory and anti-angiogenic effects, confirming the beneficial properties of mineral water sulfur components on psoriatic lesions. H<sub>2</sub>S sources seem to counteract the inflammatory processes both in arthritic fibroblast-like synoviocytes and chondrocytes, and in OA chondrocytes. All these findings provide new information about the anti-inflammatory, antioxidant, and anti-catabolic properties of H<sub>2</sub>S. In particular, H<sub>2</sub>S seems to act as a chondroprotective agent by regulating relevant factors implicated in OA pathogenesis and progression, and counteracting IL-1 $\beta$  pro-inflammatory signals that lead to cartilage destruction. The ability of H<sub>2</sub>S donors to limit the oxidative stress damage was demonstrated in cell lines of osteoblasts.

Moreover, the sulfide compounds appear to regulate inflammation and immune response in purified human peripheral blood neutrophils, eosinophils or lymphocytes. A limited number of studies attested the beneficial effect of different thermal mineral waters, in human psoriatic keratinocytes, primary neutrophils and monocytes, and OA chondrocytes.

Taken together, the *in vitro* results demonstrated anti-inflammatory and immunomodulatory effects of thermal waters and in the future they could allow to understand the specific effect of a mineral water or a particular inorganic or organic component, in terms of mechanisms of action, and to select the concentrations necessary for obtaining optimal biological benefits. However, the relevance of the preclinical tests needs to be considered with caution, since the changes that occur in cells do not necessarily reflect *in vivo* conditions.

In patients suffering from OA, *in vivo* BT has been demonstrated to be effective in having anti-inflammatory effects mediated by cytokines and changes in the circulating percentage of regulatory T cells. In low-grade inflammation-related diseases BT and mud therapy have been reported to cause a reduction in serum levels of pro-inflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$ , and regulatory cytokine IL-6, as well as an increase in anti-inflammatory growth factor IGF-1. BT and mud therapy reduce also circulating levels of other important inflammatory mediators, Prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) and leukotriene B<sub>4</sub> (LTB<sub>4</sub>) in patients with rheumatic diseases. Furthermore, the concentration of matrix metalloproteinases (MMP), involved in cartilage degradation, decreases after mud therapy in OA patients, maybe as a consequence of the reduction in pro-inflammatory mediators such as cytokines that promote MMP secretion. The reduction in pro-inflammatory mediators' concentrations after BT could be associated with the analgesic effects of the treatment, demonstrated by the concomitant reduction in pain perception. The pro-inflammatory cytokines can directly affect neuronal activity in the central and in the peripheral nervous system and can promote the production of other mediators related to inflammation and pain, such as substance P and PGE<sub>2</sub>, contributing to hyperalgesia and allodynia. Besides, in patients with rheumatic and cardiovascular pathologies treated with BT, the C-reactive protein (CRP) levels, which rise in response to inflammation, decrease.

Cellular immune response also participates in the beneficial effects of balneotherapy, for example OA patients presented an increased neutrophils' levels circulation and functional capacity, after mud therapy. In addition, changes in the percentage of circulating regulatory T cells can be implicated in the anti-inflammatory effect of BT.

In AS patients whole-body hyperthermia and speleotherapy results in changes of the proinflammatory cytokine network.

Balneotherapy could be an effective choice for the treatment of patients with FMS relieving the clinical symptoms and influencing the inflammatory mediators.

In conclusion, studies on *in vitro* samples could open the way to the scientific progress in the field of BT and future clinical studies and RCT could help to identify, in real life and in clinical practice, a personalized BT approach for each pathological condition and for each patient.

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Balneology is a medical science included by the World Health Organization (WHO) in the field of complementary and integrated medicine that uses natural methods of treatment, prevention and rehabilitation. . The recent COVID-19 pandemic has posed important scientific questions, to date only partially clarified. One of the aspects that have emerged is the fundamental role of the immune system in prevention, therapy and rehabilitation related to the COVID-19 infection.

FEMTEC has therefore questioned the role of balneotherapy in improving human immune function. The Federation has therefore decided to release a document to provide reliable scientific data based on specific methodological criteria on the role of balneotherapy in human health and, more specifically, in improving the immune response.

The Authors, Dr. Maria Chiara Maccarone, Dr. Giacomo Magro and Dr. Anna Scanu, coordinated by Prof. Stefano Masiero, Director of the Rehabilitation Department of the University of Padua (Italy) and President of the Femtec Commission for Physical Therapy, have carried out research and critical analysis of the existing literature on the subject from which emerges a picture of undoubted interest that not only seems to confirm the important role of balneotherapy in the prevention of certain diseases, but also opens interesting perspectives in other fields of research.

In this paper 30 in vitro studies on human and animal samples published between 1997 and 2020 on the effects of balneotherapy on the immune system in models of dermatological, musculoskeletal and inflammatory diseases have been considered. In addition, 10 clinical studies on healthy populations and patients suffering from arthrosis, ankylosing spondylitis, fibromyalgia and psoriasis have been considered.

On human in vitro samples, sulphurous compounds contained in thermal waters have been shown to exert an anti-inflammatory action on psoriatic lesions and artrocytic chondrocytes.

Also in vivo, in the cohorts of patients with considered osteoarthritis, balneotherapy has been shown to have anti-inflammatory efficacy, modulating the cytokinic response and modifying the percentage of regulatory T cells in the bloodstream.

In conclusion, studies on in vitro samples could pave the way for scientific progress and future clinical studies could help to identify, in real life and in clinical practice, the effects on the immune system of balneology, further developing this possibility of use of spa treatments.

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