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Original Research

The administration of the nutraceutical compound Migratens® modulates the immune response in individuals with fibromyalgia

Chiara Rizzo, MD[1#]

Lidia La Barbera, MD[1#]

Marianna Lo Pizzo, BSc, PhD[2#]

Bartolo Tamburini, BSc[2]

Federica Camarda, MD[1]

Giulia Grasso, MD[1]

Giuliana Guggino, MD, PhD[1*]

[#]Authors contributed equally to this work and share first authorship.

[1] Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, Rheumatology Unit – "P. Giaccone" University Hospital, University of Palermo, Palermo, Italy

[2] Central Laboratory of Advanced Diagnosis and Biomedical Research (CLADIBIOR), University of Palermo, Palermo, Italy

*Corresponding author
Prof. Giuliana Guggino
University of Palermo
PROMISE Department
giuliana.guggino@unipa.it

Abstract

Objectives: The aim of this study was to assess the response to Migratens® (α-lipoic acid, magnesium bisglycinate, l-tryptophan, vitamin D3, vitamin B2, coenzyme Q10, niacin) in both innate and adaptive immune cells in individuals affected by fibromyalgia (FM).

Methods: Twenty individuals with FM were enrolled in the study and were treated with Migratens®. Blood samples were collected before and after 3 months of treatment. Cytokine production and cell frequency of T helper (Th), γδ T cells and Mucosal-associated invariant T (MAIT) cells were evaluated by flow cytometry. Participants underwent complete clinical evaluation at each timepoint and correlations with clinical scores of disease activity were performed.

Results: Participants treated with Migratens® showed a significant improvement in all clinical scores evaluated. In participants treated with the nutraceutical compound Migratens®, a reduction in IFN-γ spontaneous release from both adaptive and innate T cells was evidenced. IL-9 from Th, and to a lesser extent from γδ T cells, was also significantly reduced, while IL-4 from Th increased following treatment.

No important effects were highlighted for IL-17, IL-5 and IL-13 release.

Conclusions: The administration of Migratens® to individuals with FM seems to modulate the production of cytokines through a reduction in proinflammatory molecules, such as IFN-γ and IL-9, and a concomitant increase in anti-inflammatory agents, such as IL-4, thus helping to restore the homeostasis of the immune system in FM. Our results suggest that the nutraceutical approach with Migratens® could be a viable and effective treatment in the complex management of individuals with FM.

Keywords: Fibromyalgia, nutraceutical, pain, immune response, T lymphocytes

Introduction

Fibromyalgia (FM) is a chronic syndrome characterized by widespread pain, fatigue, sleep disturbances and cognitive impairment. It is the second most common rheumatic disorder after osteoarthritis, and its overall prevalence in the general population worldwide reaches 2–3%, with a significant predominance in women [1]. The disorder impacts physical and mental functioning and the consequent deterioration in quality of life makes FM an emerging problem for health services in many countries. The involvement of proinflammatory cytokines (mainly IL-1, IL-6 and IL-8) have been described in the pathogenesis of FM and an expansion of Th1 lymphocytes, coupled with increased expression of Th1-related cytokines, TNF-α and IFN-γ, has been reported in individuals with FM [2], but few data are available on their specific role. Currently, FM therapy is based on a multi-modal treatment approach that combines rehabilitation programs with psychological and pharmacological interventions aimed at controlling pain and empowering mental and physical conditions [3,4]. Complementary and alternative treatments are also often used, including nutraceutical supplements as an "add on" therapy [5]. Clinical data on the administration of natural products in supporting FM therapy has showed promising results, although more studies with adequate methodological quality are necessary to investigate the efficacy and safety of these compounds in FM. In the present study, we have characterized cytokine production by innate and adaptive immune cells in the peripheral blood of individuals with FM and its modulation by Migratens® (α-lipoic acid, magnesium bisglycinate, l-tryptophan, vitamin D3, vitamin B2, coenzyme Q10, niacin), to better elucidate the potential pathogenetic mechanisms underlying the disorder.

Materials and methods

Participants

Twenty newly diagnosed individuals (18 females and 2 males, mean age 42 ± 7 years) with primary FM, fulfilling the 2010 ACR classification criteria [6]. At baseline (T0) participants were diagnosed and started the administration of the nutraceutical compound Migratens® (twice a day). The main demographic and clinical feature at T0 are detailed in **Table 1**. Exclusion criteria were concomitant enrolment in other clinical studies, allergies/intolerance to components of Migratens®, severe psychiatric or neurological disorders, and current pregnancy. No other pharmacological treatments were allowed during the observational period.
All enrolled individuals were evaluated at T0 and at T1, 3 months after the administration of Migratens®. At each time point, peripheral blood samples were collected and a complete clinical assessment was performed.
The present study complies with the Declaration of Helsinki, was approved by the local Ethics Committee and written informed consent was obtained from all participants.

Participants with FM *(n = 20)*	
Mean age, years (SD)	42 ± 7
Female sex, n (%)	18 (90)
Pain (VAS 0-10)	7.7 ± 1.4
Fatigue (VAS 0-10)	7.4 ± 1.4
FIQ-R (0-100)	70.7 ± 10.8
PDS (0-31)	24.6 ± 3.9

FM = fibromyalgia; FIQ-R = Fibromyalgia Impact Questionnaire-Revised; PDS = polysymptomatic distress scale; SD = standard deviation; VAS = visual analogue scale

Clinical evaluation

At each timepoint, changes in pain and in quality of life were assessed through: the visual analogue scale (VAS 0–10); the Fibromyalgia Impact Questionnaire Score-Revised (FIQ-R), final score range 0–100 [7] validated for the Italian population [8]; the Polysymptomatic Distress Scale (PDS) – PDS, sum of the widespread pain index (WPI) and symptoms severity score (SSS) – final score range 0–31 [9], respectively.

Isolation, stimulation and flow cytometry of peripheral blood mononucleate cells (PBMC)

PBMC were isolated by Ficoll-Hypaque gradient separation and incubated for 24 hours at 37°C in 5% CO^2 in several conditions: a) cell culture medium alone [RPMI 1640 (Euroclone, MI, Italy) supplemented with 10% FCS and antibiotics]; b) with 1 µg/mL ionomycin and 150ng/ml phorbol 12-myristate 13-acetate (PMA) for cell activation; c) with human T cell transact (anti-CD3/ CD28 beads) (Myltenyi Biotec) for T cell activation. Monensin (10 ug/mL, BioLegend) was added after 1 hour in each culture condition. After incubation, PBMC were stained with anti-human monoclonal antibodies (mAbs) to CD3 PerCP REA 613, CD4 FITC REA623, APC M-T466 and Pe-Vio770 REA623, CD8 PE Vio 615 REA734, CD161 V450 REA631, γδ FITC REA173 and Vα7.2 PeCy7 REA179 (Miltenyi Biotec, Bergisch Gladbach, Germany) to determine the frequency of Th cells, innate immune cells, Tγδ cells and Mucosal-associated invariant T (MAIT) cells. Intracellular staining was performed using mAbs to IFN-γ FITC REA600, IL-4 PeCy7 REA895, IL-5 APC REA1025, IL-9 APC REA1038, IL-13 PE Jes10-5A2.2 and IL-17 PE REA1063(Miltenyi Biotec, Bergisch Gladbach, Germany). Stained cells were acquired on the FACSAria flow cytometer. At least 100,000 cells (events) were acquired for each sample. Flow Cytometry Standard (FCS) files from FACSAria flow cytometer were analysed using FlowJo software (Treestar Inc Ashland, OR).

Statistical analysis

GraphPad Prism V.9 (GraphPad, San Diego, California, USA) software was used to analyse data. Comparisons between variables were performed by the non-parametric One sample T test. P values <0.05 were considered statistically significant.

Results

IFN-γ production by Th cells, T cytotoxic cells and innate immune cells decreased after Migratens® administration

To assess if the production of IFN-γ could be affected by the administration of the nutraceutical compound Migratens®, we evaluated the ability to release IFN-γ by Th cells, cytotoxic T cells $CD3^+$ $CD4^-$ and innate cells $CD3^-$ $CD4^-$ in individuals with FM at two timepoints: T0 and T1 (3 months after Migratens® administration). Our results showed a decrease of the spontaneous production of IFN-γ by Th cells at T1 compared to T0 (**Fig. 1A**), while after *in vitro* stimulation with ionomycin PMA or CD3/CD28 activation beads, IFN-γ production by Th cells remained unchanged despite the administration of Migratens® (**Fig. 1B**). Analysis of the expression of IFN-γ by $CD3^+$ $CD4^-$ T cells revealed a significant reduction of spontaneous IFN-γ production at T1, while no changes were detected after *in vitro* stimulation with ionomycin PMA or CD3/CD28 activation beads (**Fig. 1C, D**). Spontaneous release of IFN-γ by the innate immune population $CD3^-$ $CD4^-$ showed a decreasing trend; no variation between T0 and T1 was observed after the *in vitro* stimulation with ionomycin and PMA (**Fig. 1E, F**).

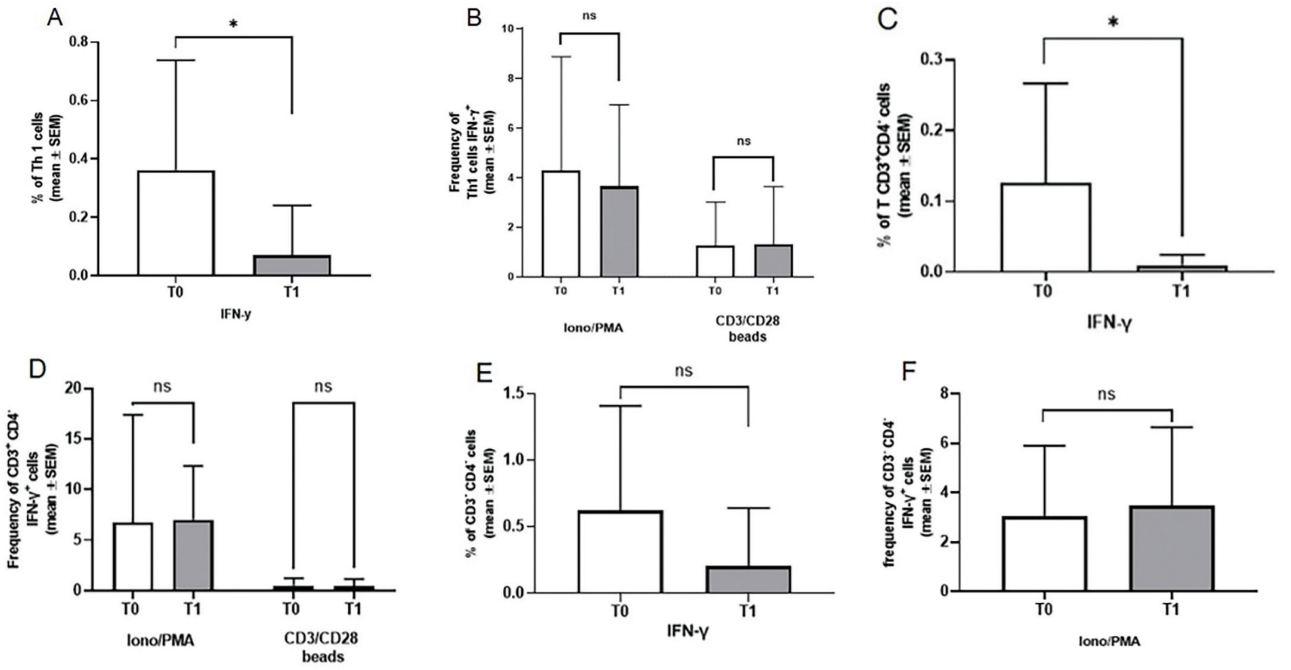

Figure 1 Effect of Migratens® on IFN-γ production
IFN-γ production by Th1 cells in the absence of stimulation (A) and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (B); by $CD3^+CD4^-$ cells in absence of stimulation (C) and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (D); by $CD3^-CD4^-$ cells in absence of stimulation (E) and after *in vitro* stimulation with iono/PMA (F), at T0 and T1 *$p<0.05$
Iono/PMA = ionomycin PMA; PMA = phorbol 12-myristate 13-acetate; Th = T helper

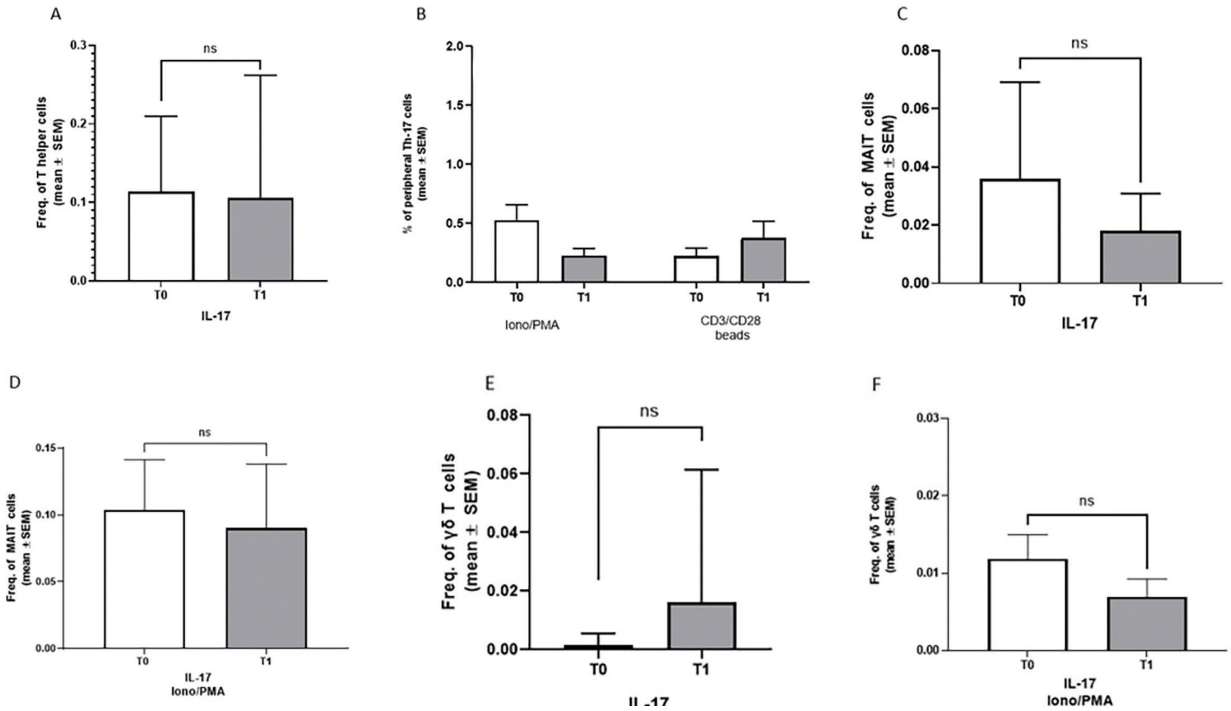

Figure 2 Effect of Migratens® on IL-17 production
IL-17 production by Th cells in absence of stimulation (A) and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (B); by MAIT cells in absence of stimulation (C) and after *in vitro* stimulation with iono/PMA (D); by γδ T cells in absence of stimulation (E) and after *in vitro* stimulation with iono/PMA (F), at T0 and T1
Iono/PMA = ionomycin PMA; MAIT = Mucosal-associated invariant T; PMA = phorbol 12-myristate 13-acetate; Th = T helper

IL-17 production by Th cells and innate-like T cells was not affected by Migratens® administration

IL-17 production by CD4+ T cells, MAIT cells and γδ T cells was also assessed in participants after the administration of Migratens®. No significant differences were observed in IL-17 production by Th cells at T0 vs T1 (**Fig. 2A, B**). IL-17 production by MAIT cells and γδ T cell population was not affected by the administration of the nutraceutical compound (**Fig. 2C, D, E, F**).

Migratens® modulated the frequency of IL-9 producing Th and γδ T cells

Th cells spontaneously produced more IL-9 than γδ T cells in individuals with FM. After treatment with Migratens®, the frequency of IL-9 producing Th was significantly reduced; whereas only a trend towards reduction was evidenced for γδ T cells. When stimulated *in vitro* with CD3/CD28 activation beads, IL-9+ Th frequency increased; after treatment their reduction was strongly significant, even in the presence of stimulation. In parallel, a trend towards a reduction in IL-9 production from Th was noted in the presence of stimulation with ionomycin PMA. γδ T lymphocytes in individuals with FM were stimulated with ionomycin PMA and the frequency of IL-9+ cells increased at T0. At T1, although not significant, a down-regulation of IL-9+ γδ T cells was evidenced (**Fig. 3**).

Among Th2 cytokines, Migratens® increased IL-4 production from Th cells

The evaluation of IL-4, IL-13 and IL-5 production from Th showed a significant increase only for IL-4 producing Th induced after treatment with Migratens®, in the absence of any stimulation. The *in vitro* stimulation with anti CD3/CD28 activation beads or ionomycin-PMA did not affect the production of IL-4 and IL-13, even after Migratens® administration. Following incubation with CD3/CD28 activation beads, an increase in IL-5 production was noted at T0; however, at T1 no differences in the frequency of IL-5+ Th were outlined (**Fig. 4**).

Effect of Migratens® on symptoms, severity and impaired functions of FM

The effect of Migratens® administration on participants' quality of life was assessed by the evaluation of changes in VAS pain, VAS fatigue, FIQ-R and PDS. Evaluating the clinical response, individuals with FM who were treated with Migratens® showed a marked improvement in all four areas from T0 to T1 (**Fig. 5**).

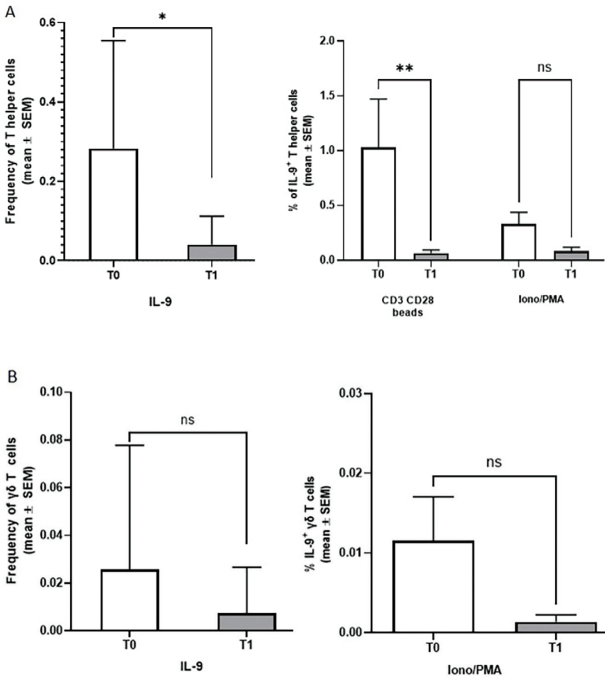

Figure 3 Effect of Migratens® on IL-9 production
IL-9 production by Th cells in the absence of stimulation and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (A); IL-9 production by γδ T cells in the absence of stimulation and after *in vitro* stimulation with iono/PMA (B), at T0 and T1 *p<0.05, **p<0.01
IL = interleukin; Iono/PMA = ionomycin PMA; PMA = phorbol 12-myristate 13-acetate; Th = T helper

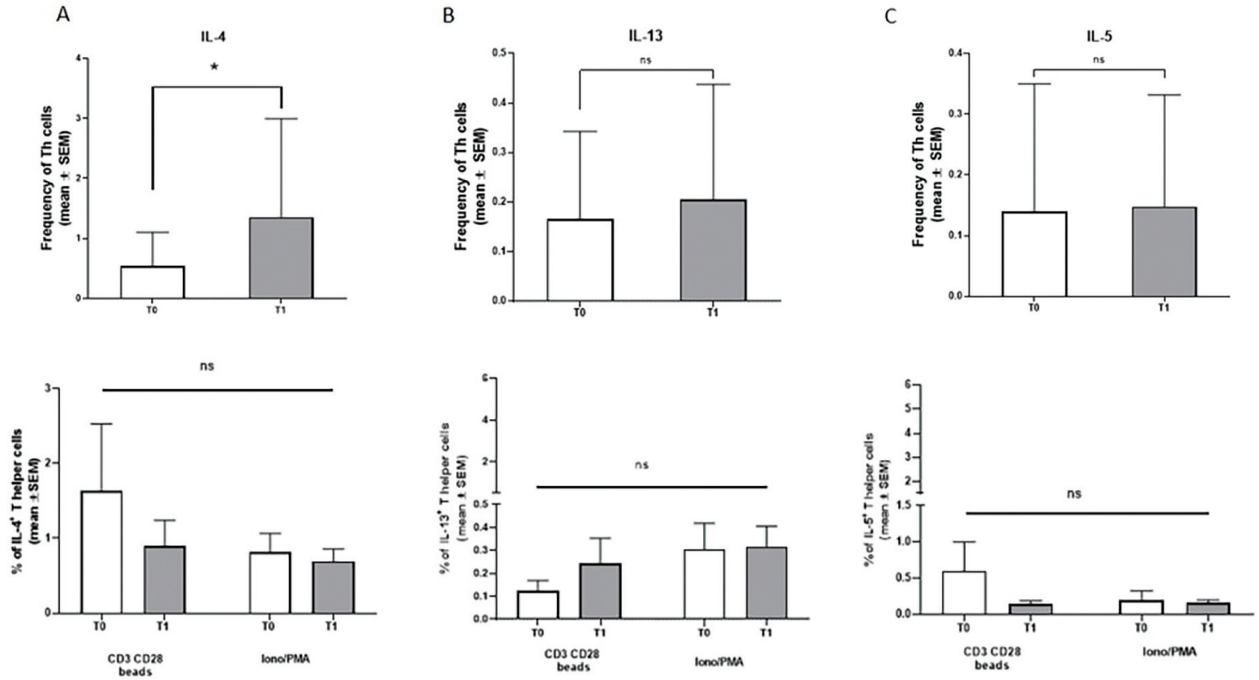

Figure 4 Effect of Migratens® on IL-4, IL-13 and IL-5 production
IL-4 production by Th cells in the absence of stimulation and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (A); IL-13 production by Th cells in the absence of stimulation and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (B); IL-5 production by Th cells in absence of stimulation and after *in vitro* stimulation with iono/PMA or CD3/CD28 activation beads (C), at T0 and T1 *p<0.05
IL = interleukin; Iono/PMA = ionomycin PMA; PMA = phorbol 12-myristate 13-acetate; Th = T helper

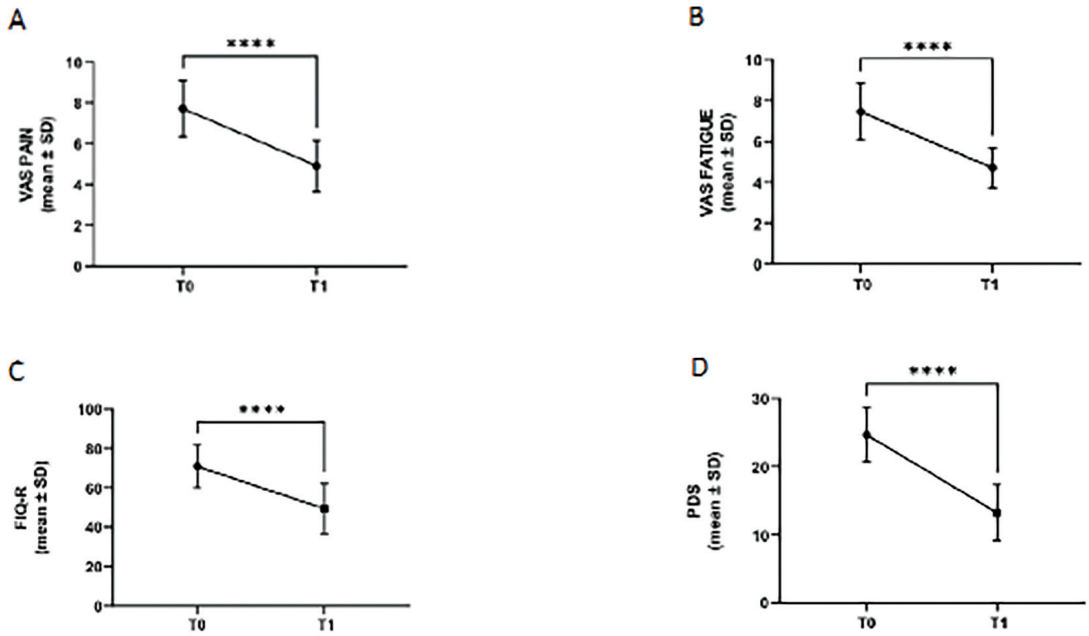

Figure 5 Effect of Migratens® administration on FM clinical evaluation
VAS pain (A), VAS fatigue (B), FIQ-R (C) and PDS (D) at T0 and T1.
Data are expressed as mean ± SD ****p< 0.0001; *p<0.05
FIQ-R = Fibromyalgia Impact Questionnaire Score-Revised; FM = fibromyalgia; PDS = Polysymptomatic Distress Scale; SD = standard deviation; VAS = visual analogue scale

Discussion

FM treatment remains a major challenge for rheumatologists. The most recent FM treatment guidelines recommend that pharmacological therapy should be considered only as an adjunctive treatment to non-pharmacological interventions in FM [10]. Indeed, some individuals with FM discontinue drugs because of side-effects or lack of efficacy, with a consequent severe impact on their quality of life [11]. Alternative medications, such as nutraceuticals, are characterized by a better safety profile and show higher acceptability when compared to standard medications [12]. Previous data described the positive effect of Migratens® administration in FM and the authors concluded that the clinical benefit observed was attributable to the nutraceutical itself [13] fatigue, altered sleep, and cognitive disturbances. The purpose of this study was to compare two alternative treatments (nutraceutical and acupuncture. To date, several studies on the compounds contained in Migratens® have been published, mainly accounting for a role in reducing oxidative stress and inhibiting the activation of proinflammatory pathways related to NF-kB and inflammasome activation [14–16]. Given the effectiveness of Migratens® in FM, we investigated, for the first time, the impact of this compound on the immune system, specifically studying innate and adaptive immune cells, and demonstrated the contribution of Migratens® in modulating cytokine production from Th and unconventional T cells, such as γδ T and MAIT cells. In FM the dysregulation of the immune system response mainly relies on a strong Th1 polarization that drives the overexpression of proinflammatory cytokines, such as TNF-α, and IFN-γ, which were all found in increased levels in plasma as well as in tissue specimens of individuals with FM. The Th1 signature of FM is intimately linked to the development of symptoms that include fatigue, fever, sleep disorder, pain and myalgia [2]. Beyond classical Th1 responses, a putative role for IL-9, a renown proinflammatory cytokine involved in several rheumatic conditions, has been recently outlined in FM [17]. The differentiation of $CD4^+$ T cells towards Th9 highlights their remarkable plasticity as they can react to environmental triggers to mount complex immune responses and, in the case of activated aberrant pathways, may determine the expansion of pathogenic cell subsets. Our results corroborate the overexpression of IL-9 in FM and the important contribution of Migratens® in modulating it. Interestingly, even γδ T cells, an important source of IL-9, partially reverted the production of IL-9. No significant effects were detected for IFN-γ and IL-17 levels. Taken together, our data point out a possible more selective mechanism of the compounds included in Migratens® to interfere with IL-9 producing cells that differs from the previously described pleiotropic effect of different interventions, such as hyperbaric oxygen therapy (HBOT) [2].

The cytokine landscape in FM is far more complex than previously believed. In particular, the reduction of anti-inflammatory cytokines, mainly IL-4, IL-5, and IL-13 [4,5], acts synergistically with the increase in proinflammatory mediators to determine the occurrence of a severe disorder characterized by widespread pain, hyperalgesia and central sensitization [18,19].

Our results suggest that Migratens® may significantly enhance IL-4 production with an unremarkable effect on IL-13 and IL-5 in individuals with FM. The role of IL-4 in pain generation was evidenced in animal models of disease, such as IL-4 deficient mice, that develop spontaneous hyperalgesia [20]. Furthermore, individuals with FM have low serum levels of IL-4 and polymorphisms of IL-4 gene are risk factors for developing FM [21]. Interestingly, IL-4 exerts analgesic functions through the inhibition of proinflammatory cytokines release that drive peripheral and central sensitization. In addition, IL-4 can

pass the blood-brain barrier and enhance neuronal survival by activating microglial cells, further contributing to protection from central sensitization. In light of this, the reduction of pain outlined in previous research on Migratens®, may be related to an increased concentration of IL-4, as demonstrated in our work; similar results were obtained with other molecules, such as ethosuximide, sodium butyrate and pregabalin [22], paving the way for possible targeted analgesic combination strategies in FM.

Clinical data corroborated the laboratory findings, showing an improvement in all evaluated parameters and suggesting that the immune system modulatory effect induced by Migratens® may be related to the alleviation of FM symptoms.

Despite the relevant results outlined, our study presents some limitations. First, the sample size is too small to draw definitive conclusions; second, we did not include a control group that would have clarified the specific role for Migratens® in modulating the immune response in FM. However, the use of a placebo was not considered ethical and some previous data account for a similar role using ancillary treatments, such as HBOT or acupuncture [2,23]. We chose to focus exclusively on Migratens® administration in a population of individuals with FM who were not treated with other drugs to reduce confounding factors.

Notably, our results provide the first evidence for the effect of Migratens® on immune cells in FM, specifically highlighting a role in affecting T cell cytokine production of both innate and adaptive immune responses. In addition, as our participants were all newly diagnosed and naïve to other treatments, we can speculate that the early use of Migratens® may concur to a reduction of the aberrant cytokine production and possibly drive a milder disorder course. We plan to better explore the impact of Migratens® in FM evolution by further studies with a long-term follow up.

Conclusion

The present study suggests that the effectiveness of a nutraceutical approach using Migratens®, a composite product of several nutraceutical principles, has a significant biological rationale related to the possible rebalance of the immune system in individuals with FM. Our data support the early use of Migratens® as part of the polymodal treatment prescribed.

Author contribution

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Chiara Rizzo, Lidia La Barbera, Federica Camarda and Giulia Grasso. Laboratory experiments were performed by Marianna Lo Pizzo and Bartolo Tamburini. The first draft of the manuscript was written by Chiara Rizzo, Lidia La Barbera and Federica Camarda and all authors commented on previous versions of the manuscript. Final revision was performed by Giuliana Guggino, Chiara Rizzo and Lidia La Barbera. All authors have approved the final version of the manuscript.

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None.

Data availability

All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to partici-

pate: The present study complies with the Declaration of Helsinki and was approved by the local Ethics Committee Palermo 1 (n. 02/2018). Written informed consent was obtained from all participants.

Conflict of Interest

Authors declare that they have no competing interests.

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