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Medical Bulletin

BC Division of Blue Cross Laboratories

From the Medical Desk of Blue Cross Laboratories, Mumbai!!!

Dear Friends,

Greetings!!!

At the outset, I take this opportunity to extend our gratitude for the tireless work that you all have been doing in these unprecedented times and serving the humanity selflessly.

I thought of connecting with you all for an update on (MEFTAL) which contains Mefenamic acid.

We are aware that Mefenamic acid has anti-pyretic, analgesic and anti-inflammatory actions, as a result of inhibition of Prostaglandin synthesis along with blocking its receptors. The efficacy and safety in this regard is very well established and has been prescribed world over for more than five decades with confidence.

Evidence now show Mefenamic acid also has a unique action of inhibiting a protein named **NLRP3 inflammasome, which on its aberrant activation is responsible for the excess release of pro-inflammatory cytokines IL-1ß& IL-18, which in turn increase the cytokines IL-6 and TNF-α, implied in the hyper-immune response of covid.**

Herein, I am glad to share that after several round of deliberations and bringing their own clinical experience to the fore, of using Mefenamic acid in Covid-19 patients, several experts have issued a consensus statement published in the Journal of Indian Medical Association, repurposing Mefenamic acid in all the three phases of Covid-19 infection viz. Viremic phase, Hyper-immune phase and Post Covid Myalgia phase. Mefenamic acid has been used by these experts in the dosage of 500 mg three times a day upto 3 months in post covid myalgia.

To summarise, Mefenamic acid has an inhibitory action on the NLRP3 inflammasome which no other NSAID has. Mefenamic Acid also has better antipyretic action than paracetamol especially during the inflammatory phase. Additionally antiviral action also has been identified, which may require further explorations.

This medical bulletin is an excerpt based upon the published literature and is brought to you as a special edition. Hope you will enjoy reading this special edition of MEFTAL and any further queries or feedback can be communicated to us to the details as given below.

STAY SAFE AND TAKE CARE!!!

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EXPERT PANEL RECOMMENDATION: USE OF MEFENAMIC ACID (MEFTAL) AS ADJUNCT IN THE TREATMENT OF COVID-19.

INTRODUCTION:

Coronavirus disease 2019 (COVID-19) pandemic has identified and experimented many drugs in its management and these agents included both new and old drugs. With the ever increasing knowledge about the pathogenicity of the disease, many different therapeutic targets are being explored, and drugs acting on these areas are repurposed for COVID-19 treatment. Hyper-inflammatory response has been one of the significant features driving the criticality of the patient's condition because of the resulting damage to the airways and several other body organs.

In this expert round table discussions, Mefenamic acid was considered for repurposing in the management of COVID-19, because of its established antipyretic, anti-inflammatory, and antiviral properties.

BASED UPON EVIDENCES AND CLINICAL EXPERIENCES:

Several rounds of discussions ensued with the Confederation of Medical Associations of Asia and Oceania (CMAAO) group of experts, the Indian Medical Association (IMA) panel, and the committee of experts, including clinicians treating or undertaking research in COVID-19 management, which was specially formed to delve into the available evidence and relevant clinical experience on Mefenamic acid.

Basis the consensus, this expert panel has recommended the use of Mefenamic acid in the various phases of COVID-19 management viz. the viremic phase, inflammatory phase and post covid phase.

IMMUNOPATHOPHYSIOLOGY OF COVID-19:

The virus and its entry into host cell-

In humans, the SARS-CoV-2 showed high transmissibility affecting large number of population but lesser mortalities as compared to MERS and SARS-CoV-2.

Coronaviruses possess single-stranded positive RNA, spike-S, membrane-M, and envelope-E proteins in the virus envelope. Nucleocapsid-N is present inside the virion that covers the RNA.

Protein S contains the receptor-binding domain for the ligand on the host cell membrane and the epitopes recognized by T and B cells, which trigger antibodies' production. The primary receptor for the SARS-CoV-2 on the host membrane is the angiotensin-converting enzyme 2 (ACE2). It is present on the membrane of many cells, including type I and II pneumocytes, small intestine enterocytes, kidney proximal tubule cells, the endothelial cells of arteries and veins, and the arterial smooth muscle. Receptor-binding domain essential for ACE2 binding mobilises conformational changes on S leading to cleavage of S1 and S2 proteins, mediated by the transmembrane serine protease 2 (TMPRSS2), enabling S2 to facilitate the fusion of the virus envelope with the cell membrane thus permitting viral entry into the cytoplasm of the host cell. Inside the cell, viral RNA serves as a template for the translation of the polyproteins leading to a reshuffling of the membranes to form the vesicles where viral replication and transcription complexes are secured. The virions are gathered in the ER-Golgi, and the secretory pathways eventually release mature virions.

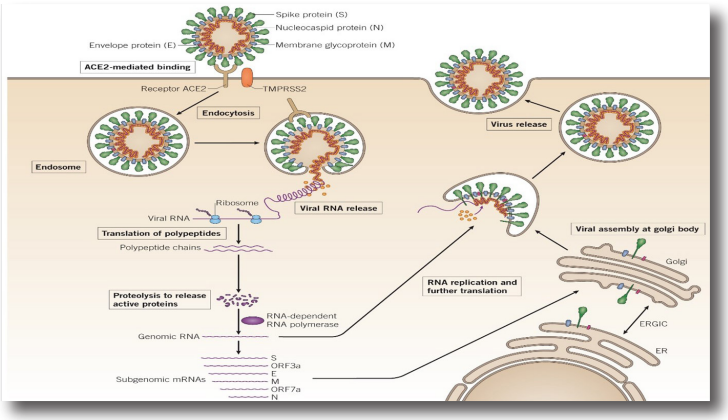

Diagram 1: SARS-CoV-2 viral lifecycle

INNATE IMMUNE RESPONSE:

SARS-CoV-2 is recognized by pattern recognition receptors (PRRs), which recruit adaptor proteins viz.interferon regulatory factor (IRF), nuclear factor kappa B (NF-kB), and activator protein-1 (AP-1) leading to the production of type I and type III antiviral interferons (IFNs) and different chemokines. These chemokines attract more innate response cells, polymorphonuclear leukocytes, monocytes, NK cells & dendritic cells. Cytokines like IL-6, IL-1ß, tumour necrosis factor (TNF-α), MCP1, CXCL1,

CXCL5, and CXCL10/IP10 are induced by SARS-CoV-2 and these findings have been supported by the increased serum levels of these cytokines in COVID-19 patients.

ADAPTIVE IMMUNE RESPONSE:
The protective response is T cell-dependent, with CD4 helping B cells help in producing specific neutralizing antibodies and cytotoxic CD8 cells eliminate infected cells. It has been found that in COVID-19, 80% of the infiltrating cells are CD8. In the case of a dysfunctional response, an exacerbated inflammatory response leads to a cytokine storm, clinically manifested by severe acute respiratory distress syndrome (ARDS) and systemic results like disseminated intravascular coagulation. The disease is hypothesized to be divided into two phases; an early phase dependent on viral replication and a later viral-independent, immune-dependent phase accompanied by an exacerbated inflammatory component.

INVOLVEMENT OF PROSTAGLANDINS:
Infection with SARS-CoV-2 ligates various pathogen recognition receptors such as TLR and/or RLRs and triggers transcription factors such as IFN regulatory factor 3 (IRF3) and NF-kB that are responsible for the expression of type I and III IFNs and pro-inflammatory mediators, including TNF-a, IL-6, and PGE2, respectively. NF-kB is the vital transcription factor responsible for the induction of pro-inflammatory cytokines. Activation of NF-kB can stimulate gene expression of inducible COX-2 and microsomal prostaglandin E synthase-1 (mPGES-1) in many cell types bringing about the production of COX-2-dependent PGE2. In humans, PGE2 promotes IL-1ß-dependent production of IL-6, macrophage colony-stimulating factor (M-CSF), and vascular endothelial growth factor (VEGF) from human fibroblasts via EP4 receptors. It also enhances induction of IL-6 and other pro-inflammatory cytokines upon many stimuli in monocytes, macrophages, fibroblasts, and airway epithelial cells through both EP2 and EP4 receptors. Besides, IL-6 also up-regulates COX-2 gene expression and increases PGE2 production and in addition PGE2 can also trigger IL-6 production in a paracrine way creating a vicious cycle.

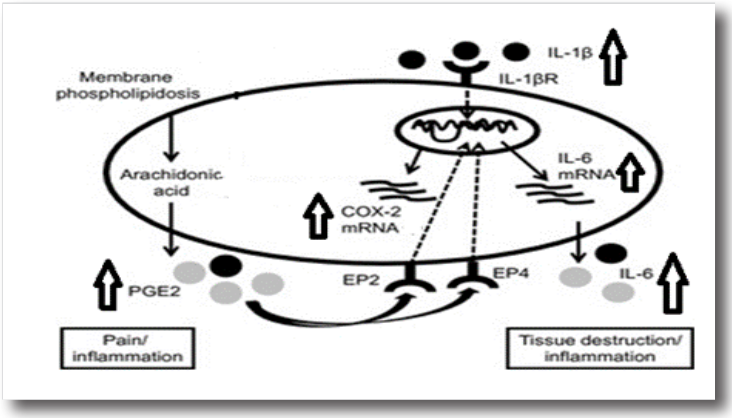

Diagram 2: Effect of PGE2 and IL-6

NLRP3 INFLAMMASOME:
It has been observed that several external and internal stimuli, including viral RNA (E protein and ORF3a of SARS-CoV-2), trigger the activation of NLRP3 inflammasome through mechanisms including formation of pores with ion redistribution and lysosomal disruption, leading to inflammation and associated cell death. Once NLRP3 is activated, procaspase-1 is converted to the active effector protease caspase-1, which leads to cleavage and maturation of pro-inflammatory cytokines like pro-IL-1ß into its active form IL-1ß and that of IL-18. These pro-inflammatory cytokines stimulate a cascade of other downstream mediators of inflammation, including IL-6, TNF-α, prostaglandins, and leukotrienes.

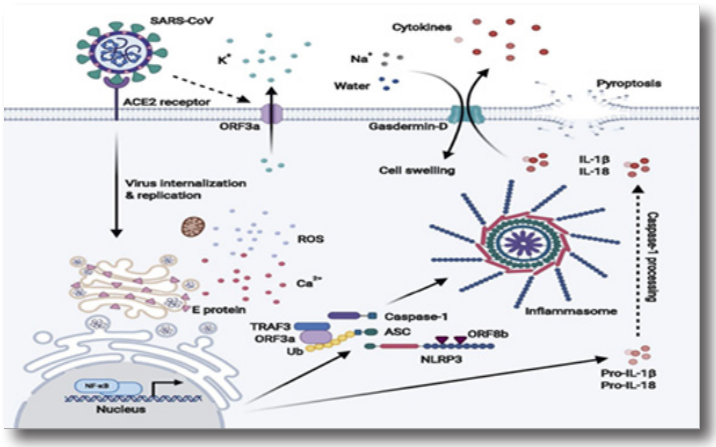

Diagram 3: SARS CoV-2 and NLRP3 Inflammasome activation

Abnormal activation and triggered cascade of downstream mediators may lead to pathological tissue injury during infection, as is seen in the case of SARS-CoV-2 infection. Infection with SARS-CoV-2 notably induces a storm of pro-inflammatory cytokines includ-

-ing IL-1ß, IL-6, and TNF-α, all of which play a vital role in the progression of tissue inflammation causing ARDS and eventually leads to death. Based on this strong inflammatory ability of the NLRP3 inflammasome, it forms an important target in the treatment strategy of COVID-19.

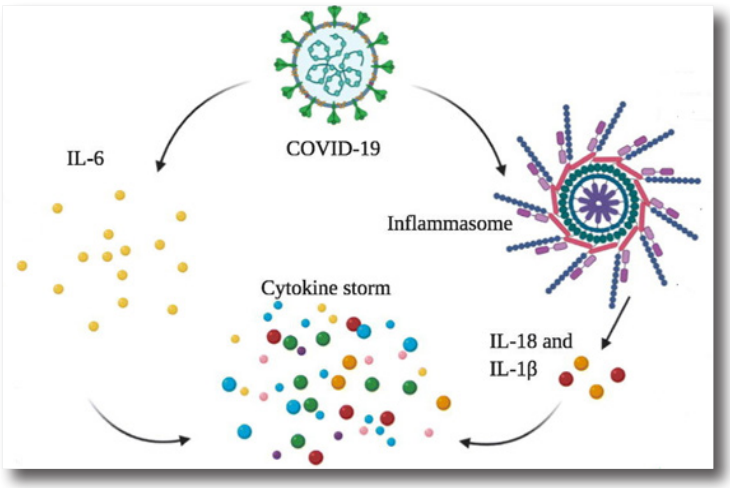

Diagram 4: Inflammasome ,pro-inflammatory cytokines and cytokine storm in Covid-19

CYTOKINE STORM AND COVID-19:

Mortality in COVID-19 patients has been associated with the presence of the "cytokine storm" induced by the virus and the hyper-inflammatory immune response of the host. Excessive formation of pro-inflammatory cytokines causes aggravation of ARDS and widespread tissue damage resulting in multi-organ failure and death. The cytokine storm in COVID-19 is related directly to lung injury, multi-organ failure, and unfavourable prognosis of severe COVID-19. Three of the most critical pro-inflammatory cytokines of the innate immune response are IL-1ß, TNF-α, and IL-6. The increase in cytokine levels leads to the recruitment of several immune cells like macrophages, neutrophils, and T cells from blood circulation into the infection site with the destructive effects on human tissue resulting from the destabilization of endothelial cell to cell interactions, damage of vascular barrier, capillary damage, diffuse alveolar damage, multi-organ failure, and eventual death. Lung injury is a severe manifestation of the cytokine storm that can progress into ARDS. Given the above discussion, the early detection and prompt treatment can lead to a better outcome of COVID-19.

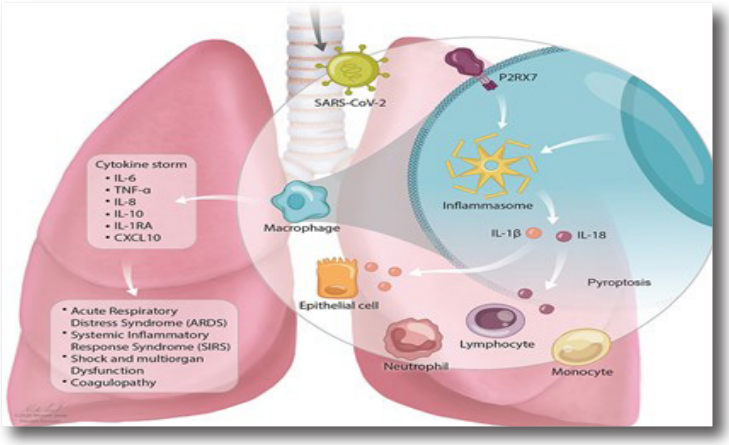

Diagram 5: Cascade of inflammatory cytokine activation leading to ARDS

FEVER IN COVID-19:

The first presentation of fever during the first week in COVID-19, during the viral phase of the illness, is probably a manifestation of the body's immune reaction to the replication of the virus to enhance immunity. However, when the infection does not resolve in due course, it leads to viral activated state of dysregulated inflammation referred to as cytokine storm. In these cases, where fever occurs due to severe inflammation, it may be counterproductive because the fever is not beneficial at this stage, & may promote inflammation leading to hyper immune activation. Hence, fever may have a differential impact on the prognosis during the viral and inflammatory stage of the disease marking the use of antipyretics in different stages of COVID-19 infection becomes imperative.

POST COVID MYALGIA:

Myalgia reflects generalised inflammation and myalgia caused by COVID-19 is more prolonged and severe when compared with other viral infections and may be unresponsive to traditional painkillers.

As the acute COVID-19 infection has been alleviated, some patients experience long-term symptoms including persistent fatigue, diffuse myalgia, depressive symptoms, and non-restorative sleep. Due to the build-up of cytokines in the central nervous system may be the cause of post-viral symptoms because of the pro-inflammatory cytokines passing through the blood-brain barrier in circumventricular organs such as the hypothalamus, leading to autonomic dysfunction.

REPURPOSING OF MEFENAMIC ACID IN THE MANAGEMENT OF COVID 19

ANTI-VIRAL ACTIVITY:

Angiotensin-converting enzyme 2 is the host cell receptor for the S protein of SARS-CoV-2, and TMPRSS2 is required for S protein priming of SARS-CoV-2. Their inhibition may prevent cell entry of the SARS-CoV-2.
Mefenamic Acid is known to inhibit serine proteases.
Earlier in-vitro and pre-clinical studies have shown that mefenamic acid possesses antiviral activity. In Chikungunya virus infections the activity of Mefenamic acid showed enhanced antiviral action when administered in combination with the common antiviral drug, ribavirin. The combination of the antiviral and anti-inflammatory effects of mefenamic acid was beneficial in significantly reducing the pathological signs. Another study in dengue infections demonstrated that Mefenamic acid, along with doxycycline, had antiviral activity.

ANTI-PYRETIC ,ANTI-INFLAMMATOY, AND ANALGESIC ACTIONS:

Cyclooxygenase inhibition-

There are two COX isoforms, COX-1 and COX-2, which catalyse the first two steps of prostaglandin biosynthesis from AA and are the pharmacological targets of NSAIDs. Mefenamic acid depicts preferential inhibition of COX-2 enzyme.

Action on prostaglandins-

It has been observed that prostaglandins (PGE2) are responsible for inflammation, pain and fever. Mefenamic acid possess dual inhibitory action; they rapidly neutralize the effects of preformed prostaglandins by blocking the prostanoid (EP) receptors and as well as by inhibiting the COX enzymes.

Action on NLRP3 inflammasome-

Only fenamate NSAIDs (Mefenamic acid) have been found to selectively inhibit the NLRP3 inflammasome and thereby IL-1ß release by blocking the membrane volume regulated anion [chloride] channel (VRAC). This blockade acts independently of its COX-mediated anti-inflammatory activity.

In the SARS-CoV-2 infection, there is a role of NLRP3 inflammasome inhibitors in terms of the inflammatory manifestations; this draws attention towards Mefenamic acid.

COMPARISON OF MEFENAMIC ACID WITH OTHER NSAIDs:

Studies demonstrated amongst all NSAIDs only fenamates (Mefenamic acid) selectively inhibits NLRP3 inflammasome. On the contrary Indomethacin and Paracetamol have shown to activate NLRP3 inflammasome causing intestinal and liver damage respectively. However in one of the study Indomethacin has been shown to have NLRP3 inhibitory action in pancreatic cells, thereby indicating its ambiguous nature. Other NSAIDs like naproxen, diclofenac, aceclofenac, ibuprofen or nimesulide had no documented inhibitory action on NLRP3 inflammasome. Additionally Ibuprofen has been observed to aggravate the SARS-CoV-2 infection.
As is clear from the discussion in previous sections, mefenamic acid is a potent COX inhibitor and causes both central and peripheral analgesic action. In a study comparing the efficacy and safety of paracetamol and Mefenamic acid in the treatment of fever, it was reported that Mefenamic acid was more effective antipyretic drug. Mefenamic acid thus exhibited a highly significant reduction in the body temperature baseline to the sixth hour compared with paracetamol. Based on the above evidence, Table 1 had been formulated to depict the effect of different NSAIDs on different aspects of the immune-pathogenesis of COVID-19.

Cytokine	Paracetamol	Naproxen	Indomethacin	Nimesulide	Mefenamic acid
IL-1β		↓ +	↓	↓	↓++
IL-6	↔	↓+	↓+	↓+	↓+++
IL-18	×	×	×	×	↓+
TNF-α	↓	↓	↔	↓	↓++
NLRP3	×	×	Ambiguity↔	×	✓
Viral load	×	Prevents viral entry & replication	Inhibits viral replication at a higher dosage	×	Inhibits the serine protease & prevents viral entry & replication

Table 1:-Action of Different NSAIDs on the Various Immuno-pathogenesis of COVID-19

EXPERT PANEL RECOMMENDATIONS:

1. COVID-19 can be classified into: a viral phase, second immune-inflammatory phase and post covid infection with myalgia phase.
2. Given the understanding of the fever in COVID-19, it is important to note that paracetamol does not possess the same anti-inflammatory features that NSAIDs possess. Experts also opined that anti-inflammatory drugs like ibuprofen could act as an aggravating factor for the infection.
3. Mefenamic acid with established antipyretic action is one such NSAID that can be used safely right from the day one of infection.
4. Mefenamic acid can be used at any stage of COVID-19, contradictory to steroids, which should be avoided during the viremic phase of the infection.
5. An additional advantage of mefenamic acid is that it possesses anti-inflammatory, analgesic, and antiviral effects as well. When used in the inflammatory phase of the disease, mefenamic acid acts on the NLRP3 inflammasome and inhibits it, thereby reducing IL-1ß, IL-18, IL-6, and TNF-α, which have been implicated as important cytokines for leading to cytokine storm.
6. In individuals with persistently high C-reactive protein (CRP) (persistent inflammation), mefenamic acid can be given in a dose of 500 mg thrice a day for long-term (up to 3 months), in post-COVID syndrome.
7. Mefenamic acid is effective in a fever, not responding to paracetamol.

The recommendations for the use of Mefenamic acid in the management of COVID-19 adult patients

1. 500 mg mefenamic acid 3 times a day for 7-10 days.
2. In case of high CRP levels persisting in the inflammatory phase, may continue with mefenamic acid (500 mg, three times a day) for up to three months or till the CRP value optimize, for managing inflammation.
3. In post-COVID syndrome, mefenamic acid may be considered in a dose of 500 mg-three times a day for up to three months.

Precautions-

In severely renal impaired cases or elderly patients with increased risk of gastrointestinal bleeding Mefenamic acid should be avoided. Mefenamic acid is pregnancy category C; it should only be used with caution if benefits outweigh risks. However, it should be avoided in the third trimester. The American academic of paediatrics endorses the use of Mefenamic acid during lactation.

Mefenamic acid is a safe antipyretic in children; however, in COVID-19, it is not advisable in children under 14 years of age due to lack of clinical evidence.

CONCLUSION:

Several targets are being explored for treating the COVID-19 infections. Initiating from inhibiting the viral entry to inhibiting the various immune-inflammatory pathways causing cytokine storm are being experimented whilst repurposing of many available drugs. IL-1ß, IL-6, and TNF-α, are the three most important pro-inflammatory markers leading to tissue inflammation, ARDS, and eventual death.

Mefenamic acid is an NSAID with antiviral, anti-inflammatory, analgesic, and antipyretic activities. It is a preferential COX-inhibitor, which also has an inhibitory action on the NLRP3 inflammasome and additionally inhibits the serine proteases of the virus. It has shown potent action in blocking all the three implicated pro-inflammatory biomarkers responsible for causing cytokine storm.

Various members from the expert panel shared their anecdotal experience on the effectiveness of mefenamic acid as an antipyretic, analgesic, and anti-inflammatory agent in the management of COVID-19 patients. Thus, the expert panel has recommended the use of mefenamic acid (500 mg, thrice a day) in the management of COVID-19 in adults. However, more extensive clinical trials are warranted to establish the same in the management of COVID-19.

SOURCE:

- Aggarwal KK,et.al; Repurposing Mefenamic acid in the management of Covid 19.JIMA.2021;119(1):16-23.
- https://www.tocris.com/research-area/covid-19-research
- Kawashima M,et.al; January 2013,Journal of Oral Pathology and Medicine 42(6)
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- Paniri A ,et.al; Life Sci. 2020 Sep 15; 257: 118114.

KEY POINTS

1. SARS-CoV-2 has high transmissibility and lower mortality as compared to SARS CoV and MERS.
2. Fever may exist during viraemic and inflammatory phases.
3. IL-6, IL-1ß and TNF-α, are important pro-inflammatory cytokines and excess of these leads to hyper immune response resulting in cytokine storm.
4. Aberrant activation of NLRP3 inflammasome can enhance release of these pro-inflammatory cytokines.
5. Fever during the inflammatory phase may not respond to Paracetamol.
6. Unlike other NSAIDs, Mefenamic acid has been found to have inhibitory action on NLRP3 inflammasome.
7. Mefenamic acid had established antipyretic, potent analgesic and anti-inflammatory actions. It has shown to have antiviral action and also potentiating action of other antiviral agents.
8. Expert panel recommends Mefenamic acid in the dosage of 500 mg thrice day for adults in all the phases of covid-19 infection viz.viraemic phase (antipyretic and antiviral actions), inflammatory phase (antipyretic & anti-inflammatory actions) and post covid myalgia (analgesic and anti-inflammatory actions) with duration ranging from 7 days to 14 days as necessitated as antipyretic and anti-inflammatory and up to 3 months in post covid myalgia.

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