has been reported as up to 9% in some studies [81]. Scrotal attacks are unilateral, self-limited, and painful with red swelling of the testicle lasting 48–72 h. The presence of high fever helps in the differential diagnosis of testicular torsion.

Albeit rare (about 13% of patients in the Yildirim G. et al. series [82]), erysipelas-like erythema (ELE) is the most typical cutaneous manifestation of FMF, seen most frequently associated with arthritis as a comorbidity. It appears as tender, indurated, inflamed, and erythematous plaques, usually located over crural areas, the ankle joint, and the dorsum of the foot. Foot erythema is usually associated with ankle arthritis. It may be triggered by physical effort and subside spontaneously within 48 to 72 h of rest. Recurrent oral ulceration was found to be relatively frequent (10%) in FMF [83]. Interestingly, heterozygous mutations in exon 2 on the phosphorylation site of pyrin (c.726C > G; p.Ser242Arg) have been associated with a unique phenotype distinct from the typical FMF, characterised by severe acne and pyoderma gangrenosum, in the so-called pyrin-associated autoinflammatory diseases with neutrophilic dermatosis (PAAND) [32].

6. Associated Diseases

Several inflammatory and autoimmune conditions have been associated with FMF and MEFV gene mutations, probably due to common dysregulations in the immune system but also to the high frequency of MEFV carriers in some populations. IgA vasculitis is the most frequent in FMF patients (with a prevalence of 2.7–7% vs. 0.003–0.026% in controls), followed by polyarteritis nodosa (PAN) with a prevalence of 0.9–1.4% (vs. 0.0005–0.0031% in the general population) [84]. The demyelinating central nervous system disease multiple sclerosis (MS) is enriched in FMF patients compared to Israeli and Turk populations [85,86]. In this regard, homozygosity for the M694V MEFV mutation may be a genetic modifier in MS, aggravating the phenotype of MS. Recently, the frequency of ankylosing spondylitis (AS), IgA vasculitis, juvenile idiopathic arthritis (JIA), PAN, and MS was found to be increased in a big cohort of patients with FMF when compared with those in the literature [87]. Finally, a strong association with hidradenitis was also shown [88].

7. Diagnostic and Classification Criteria

The first set of diagnostic criteria was created for adults by experts in Tel Hashomer Hospital [60] (Table 1), and thirty years later, they were refined by Livneh et al. [89] (Table 2). However, a diagnostic standard with high specificity and sensitivity was also necessary for the paediatric population, with the aim of an early diagnosis. Indeed, in 2009, a new set of paediatric criteria was developed by Yalçinkaya and Özen [90] (Table 3). These were validated with a cohort of Turkish children, reaching a sensitivity and specificity of 88.8% and 92.2%, respectively, and also encompassing some clinical aspects that are typical of children as opposed to adults (inability to express pain location, different range in the duration of attacks, diagnosis prior to appendicectomy, etc.).

Table 1. Tel Hashomer criteria for FMF diagnosis (adult criteria).

Tel Hashomer criteria (at least two major criteria or one major criterion plus two minor criteria)
Major criteria
- Recurrent febrile episodes accompanied by peritonitis, synovitis, and pleurisy. - AA amyloidosis without predisposing disease. - Favourable response to continuous colchicine administration.
Minor criteria
- Recurrent febrile episodes. - Erysipelas-like erythema. - FMF diagnosed in a first-degree relative.

Table 2. Livneh criteria for FMF diagnosis.

Livneh criteria (at least one major criterion, or two minor criteria, or one minor criterion plus at least five supportive criteria, or one minor criterion plus at least four of the "first" five supportive criteria)
Major criteria
- Typical attack * of generalised peritonitis. - Typical attack * of unilateral pleuritis/pericarditis. - Typical attack * of monoarthritis. - Presence of fever alone (rectal temperature of 38 °C or higher).
Minor criteria
- Incomplete attack ** involving the abdomen. - Incomplete attack ** involving the chest. - Incomplete attack ** involving one large joint. - Exertional leg pain. - Favourable response to colchicine.
Supportive criteria
- Family history of FMF. - Appropriate ethnic origin. - Age less than 20 years at disease onset. - Severity of attacks requiring bed rest. - Spontaneous remission of symptoms. - Presence of symptom-free intervals. - Transient elevation of inflammatory markers. - Episodic proteinuria or hematuria. - Non-productive laparotomy with removal of a "white" appendix. - Consanguinity of parents.

* Typical attacks are defined as recurrent (≥ three of the same type), febrile (rectal temperature of 38 °C or higher), and short (lasting between 12 h and 3 days).** Incomplete attacks are defined as painful and recurrent flares that differ from typical attacks in one or two features as follows: (1) normal temperature or lower than 38 °C, (2) attacks longer than 1 week or shorter than 6 h, (3) no signs of peritonitis recorded during an acute abdominal complaint, (4) the abdominal attacks are localised, and (5) arthritis involves joints other than those specified [86].

Table 3. Yalcinkaya–Ozen criteria for FMF diagnosis (childhood criteria).

Yalcinkaya-Ozen (childhood) criteria (at least two out of five criteria)
- Fever (axillary temperature > 38 °C, 6–72 h of duration, ≥ three attacks). - Abdominal pain (6–72 h duration, ≥ three attacks). - Chest pain (6–72 h duration, ≥ three attacks). - Oligoarthritis (6–72 h duration, ≥ three attacks). - Family history of FMF.

The paediatric criteria yielded a better sensitivity but a poorer specificity than the previous criteria when applied to an international cohort of children from either European or Eastern Mediterranean regions [91]. Conversely, the Tel Hashomer criteria displayed the best specificity but poor sensitivity. A higher specificity was meant to minimise the diagnostic failure or delay, although FMF diagnosis still needed to be refined with the inclusion of genetic data [92].

Indeed, in 2015, a group of experts built a set of evidence-based recommendations using a systematic literature review [93]. During the consensus meeting, the specialists confirmed the literature and concluded that FMF is a clinical diagnosis, which can be supported but not necessarily excluded with genetic testing (Strength B). This statement is currently a matter of debate since many authors believe that the term FMF should be applied only to patients carrying MEFV mutations (Ben-Chetrit), as FMF is a genetic condition.

In this line, the new evidence-based Eurofever/PRINTO classification criteria, developed for inherited recurrent fevers in 2019, includes for the first time an association

between clinical and genetic variables, resulting in a high sensitivity and high specificity classification tool [94] (Table 4). These criteria can be used to differentiate among different inflammatory conditions and, despite being classification criteria, are mainly used for research purposes (clinical trials, translational studies). They may provide both clinical-based and genetic-based guides useful for the diagnostic orientation of FMF in clinical practice.

Table 4. Eurofever/PRINTO classification criteria for FMF.

Eurofever/PRINTO Classification Criteria for FMF
Presence of confirmatory MEFV genotype * and at least one, or not confirmatory MEFV genotype ** and at least two, of the following:
- Duration of episodes 1–3 days. - Arthritis. - Chest pain. - Abdominal pain.

A patient with (1) evidence suggesting elevation of acute phase reactants (ESR or CRP or SAA) in correspondence to clinical flares and (2) careful consideration of possible confounding diseases (neoplasms, infections, autoimmune conditions, other inborn errors of immunity) and a reasonable period of recurrent disease activity (at least 6 months) is classified as having hereditary recurrent fever if the criteria are met. * Pathogenic or likely pathogenic variants (heterozygous in AD diseases, homozygous or in trans (or biallelic) compound heterozygous in AR diseases). ** In the trans compound, heterozygous for one pathogenic MEFV variant and one VUS, or biallelic VUS, or heterozygous for one pathogenic MEFV variant [91].

8. Interpretation of MEFV Gene Variants

In routine practice, when the patient's symptoms are consistent with the diagnosis of FMF, genetic tests are suggested. For the interpretation of variants resulting from molecular analysis, a committee has developed guidelines classifying genes as: (a) clearly or likely pathogenic; (b) variants of unknown significance or VOUS; or (c) clearly or likely benign [95].

M694V is considered a very severe mutation, and if present in homozygosity, even asymptomatic individuals should be considered for treatment (Strength A, [93]). M694V, V726A, M694I, M680I, and E148Q account for 70–80% of the cases in Mediterranean countries [96]. However, in cases where an uncommon variant is identified, physicians and molecular geneticists can utilise the INFEVER database. INFEVERS (Internet Fevers), created in 2002, is an online database that documents all the information available on mutations in autoinflammatory disorders-related genes (http://fmf.igh.cnrs.fr/ISSAID/infevers/index.php (accessed 30 April 2023)) [97]. It was conceived as a universal tool to gather and share all data on the mutational spectrum of HRF genes in a centralised location to highlight information that can be missed if reported separately. More specifically, it aims to overcome the challenges in interpreting VOUS with a low frequency which may function as susceptibility alleles to inflammation or new and rare genetic variants associated with a clear phenotype [98]. To date, this website documents more than 350 MEFV variants together with classification status (benign, likely benign, VOUS, likely pathogenic, pathogenic) and the centre that made the notification (Figure 3). The information provided in INFEVER can be complemented with other databases on the clinical significance of human genetic variants (ClinVar), and some in silico prediction tools (AGVGD, Sorts Intolerant from Tolerant, Polyphen-2, and Combined Annotation-Dependent Depletion score).

9. Treatment

9.1. Colchicine: Mechanism of Action

Colchicine is the oldest known drug still used today [99]. It is an alkaloid derivative of the plant *Colchicum autumnale*.

In the 1960s, colchicine's ability to bind microtubules was discovered, revealing its antimitotic action [100]. However, colchicine's action in FMF still remains to be elucidated to some degree. Microtubules, the molecular targets of colchicine, operate pleiotropi-

cally within the cell, governing intracellular organelle and vesicle transport, secretion of cytokines and chemokines, cellular migration and division, and regulating gene expression [101]. To do so, microtubules act in a dynamic fashion, changing their shape, by adding and losing tubulin heterodimers, in a continuous equilibrium between extension and shrinkage. Colchicine blocks polymer elongation, effectively inhibiting microtubule properties [102]. Colchicine action leads to impaired neutrophil chemotaxis, by diminishing the expression of L-selectin on neutrophils cell membranes and E-selectin on endothelial cells [103], and neutrophil function, by inhibiting superoxide production [104]. Moreover, colchicine dampens the activation of macrophages and the degranulation of mast cells [105] and interferes with TNF-α pro-inflammatory actions [106] and, thereby, with the NF-kB signalling cascade [107].

In addition to the indirect action on chemotaxis, motility, and stimulation of leukocytes, colchicine has been demonstrated to inhibit the NLRP3 inflammasome, thereby suppressing caspase-1 activation in gout [108]. Additionally, it may also have a distinct inhibitory function on the pyrin inflammasome, explaining its specific effect on FMF and not in other autoinflammatory diseases. In fact, by acting on microtubes, colchicine is thought to activate—or release from inhibition—RhoA, resulting in suppression by phosphorylation of the pyrin inflammasome assembly [19].

9.2. Colchicine: Metabolism and Toxicity

Colchicine is absorbed in the jejunum and ileum. Its bioavailability depends on the hepatic, biliary, and luminal intestinal multidrug transporter P-glycoprotein 1 (PGY 1). The altered expression of this transporter protein may signify suboptimal therapeutic effects or drug toxicity [109]. Colchicine is eliminated with biliary excretion and through the stool [110]. A significant role in colchicine metabolism is played by enteric and hepatic cytochrome P450 3A4 (CYP450 3A4), which catalyses the demethylation of colchicine into the inactive metabolites 2- and 3-demethtylcolchicine. This is relevant because drugs modifying the activity of this cytochrome can result in colchicine-induced toxicity. Finally, 20% of drug disposal is accounted for by kidney secretion.

Colchicine is a safe drug that has been used for a long time; however, it has a narrow therapeutic index and its commonest side effects may occur even at treatment dosages. These are mainly gastrointestinal: cramping, abdominal pain, hyperperistalsis, diarrhoea, and vomiting. These manifestations appear in 10–15% of patients and tend to resolve after a period of treatment or dose reduction [111]. Blood dyscrasias and neuropathies are features of a chronic-type overdose [112].

High colchicine concentrations are extremely toxic, leading to severe microtubule disarrangement. The affected cells experience a halt in protein assembly, endocytosis, exocytosis, cellular motility, mitosis, cardiac myocyte conduction, and contractility [113]. The accumulation of these mechanisms may lead to multi-organ dysfunction and failure, consisting of three overlapping phases. Around 10–24 h from ingestion, severe gastrointestinal manifestations appear. Then, 24 h to 7 days later, multi-organ dysfunction takes place: bone marrow failure, renal insufficiency, adult respiratory distress syndrome (ARDS), arrhythmias, disseminated intravascular coagulation (DIC), neuromuscular disturbances, and alopecia are seen [114]. If the patient survives, recovery occurs in a week or so [111].

Colchicine overdose can occur when daily doses are not adjusted for reduced renal function or interacting medications. Indeed, simultaneous use of CYP3A4 inhibitors/competitors, including clarithromycin and erythromycin, many HIV medications, calcium channel blockers and azole antifungals, or P-gp inhibitors/competitors such as ciclosporin and ranolazine, can increase colchicine concentration [109].

Colchicine usage has also been associated with an increase in liver function enzymes, for reasons that are not always clear.

Finally, in addition to the abdominal side effects of colchicine, evidence points toward the exitance of drug-induced lactose intolerance in treated FMF patients, which can be corrected with a lactose-free diet [115].

The risk of colchicine-driven oligo-/azoospermia is still a matter of debate. Probably, the frequency of azoospermia is influenced more by the underlying pathology [116], or by the presence of testicular amyloidosis [117], rather than the drug itself. Indeed, healthy volunteers do not experience infertility under colchicine treatment [118], and colchicine does not cause reduced sperm motility [119].

Concerning female fertility, colchicine therapy throughout pregnancy seems to carry no substantial teratogenic or mutagenic risk when used at recommended doses [120]. Additionally, colchicine was shown not to be associated with a higher rate of miscarriage, stillbirth, low birth weight, or early delivery [121].

9.3. Colchicine: Management in Familial Mediterranean Fever

Early independent RCTs demonstrated that daily colchicine is highly effective for preventing attacks in this disorder in a dose-related fashion [4,5,122].

According to the ongoing EULAR recommendations [123], the "starting dose" of colchicine was defined as $\leq$0.5 mg per day for children less than 5 years old, 0.5 mg per day for 5–10-year-old children, and 1 mg for those aged 10–18 years and for adults.

The dose should be guided mainly by the occurrence of clinical symptoms and serological inflammation, with the indication to increase the dose of 0.25 mg/day in a stepwise fashion until the maximally tolerated dose [123]. The maximal dose is considered to be 1 mg/day for children aged less than 5 years, 2 mg/day for pre-pubertal children, and 3 mg/day for post-pubertal children and adults [124].

Very few studies on colchicine dose per kilo have been completed. Using a cohort of children, the mean effective colchicine dosage was calculated to be 1.46 $\pm$ 0.41 mg/m^2/day or 0.05 $\pm$ 0.02 mg/kg/day for children <5 years; 1.19 $\pm$ 0.3 mg/m^2/day or 0.03 $\pm$ 0.01 mg/kg/day for children 6–11 years old; and 0.84 $\pm$ 0.2 mg/m^2/day or 0.027 $\pm$ 0.01 mg/kg/day for children 11–15 years old [125] (mean dose of the whole group was 1.16 $\pm$ 0.45 mg/m^2/day or 0.03 $\pm$ 0.02 mg/kg/day). These findings are consistent with a later study evaluating the influence of anthropometric parameters on colchicine dosage: young children received higher doses of colchicine according to their body weight as compared with older children. Furthermore, this analysis revealed that colchicine intake was best correlated with body surface area (~1.03 mg/m^2/day) [126].

The optimal treatment dose still remains to be defined; however, in any case, colchicine doses should not—and usually does not—reach values of 0.5–0.8 mg/kg, which are highly toxic or lethal (>0.8 mg/kg) [111].

10. Colchicine Resistance

Despite optimal treatment, around 5% of patients do not respond at all to the maximally tolerated dose of colchicine. A higher percentage (from 20 to 40%) of patients display an incomplete response, with a reduction but not complete control of fever episodes.

In 2016, the European League Against Rheumatism (EULAR), in its recommendations for the management of FMF, defined resistance as one or more attacks per month in compliant patients who had been receiving the maximally tolerated dose for at least 6 months [123]. More recently, a consensus of experts updated these recommendations with several statements, including some recommendations on adherence, dose adjustment criteria, and quality of life [127]. The conclusions of the consensus were schematically reported in seven statements (Table 5).

Table 5. The 2021 Delphi consensus final statements by Özen et al. on colchicine resistance/intolerance and their application in a national multicentre longitudinal study.

	Delphi Expert Consensus Statements (Özen et al., 2021 [124])	**Results of a National Multicentre Longitudinal Study (Bustaffa et al., 2021 [125])**
Adherence	Statement 1: Colchicine is the drug of choice for the treatment of FMF, and adherence is a critical issue. For the following statements, it is assumed that the patient is adherent to their prescribed colchicine treatment.	Overall, 83.8% displayed optimal adherence (>90% of prescription); 10.6% displayed good adherence (50–89% of prescriptions); 2.0% displayed poor adherence (<50% of prescriptions); and 3.5% displayed no adherence.
Dose adjustment criteria	Statement 2: When utilising colchicine to treat FMF, it is recommended to adjust the dose based on disease activity, with the adjustment of maximal dose for children depending on age (and weight).	The percentage of patients taking a colchicine dose without adjustments were: - Patients < 5 years: 71.3%; - Patients 5–10 years: 35.3%; - Patients aged 10–18 years: 57.0%; - Adults: 67.1%.
Recommended maximum colchicine dose	Statement 3: The maximum recommended colchicine dose for the treatment of FMF is 1–3 mg per day, depending on age and weight, limited by signs of toxicity and tolerability (see below).	No patient reached the maximum recommended dose.
Resistance to colchicine	Statement 4: For a patient receiving the maximum tolerated dose of colchicine, resistance to colchicine is defined as ongoing disease activity (as reflected by either recurrent clinical attacks (average one or more attacks per month over a 3-month period), or persistently elevated CRP or SAA in between attacks (depending on which is available locally)) in the absence of any other plausible explanation.	Resistance was be defined as the persistence of fever attacks, despite optimal treatment. Overall, 54.2% patients had a complete disease control; 30.1% patients had < one episode/month for 3 months; 8.5% had ≥ one episode/month for 3 month; and 7.2% had persisting disease with an unknown frequency of attacks.
Inclusion of secondary amyloidosis in the definition of colchicine resistance	Statement 5: AA amyloidosis develops as a consequence of persistent inflammation, which may be a complication of colchicine resistance.	Five adult patients (2.1%) displayed amyloidosis, two of which were prescribed anti-Il1 treatment.
Colchicine intolerance	Statement 6: Colchicine intolerance, which generally manifests as mild gastrointestinal symptoms (such as diarrhoea and nausea), is common but can limit the ability to achieve or maintain the effective dose. Dose-limiting toxicity is rare and may include serious gastrointestinal manifestations, such as persistent diarrhoea, elevated liver enzymes, leukopenia, azoospermia, neuromyopathy, etc.	Eight patients (3.4%) with follow-up had persistent manifestations of intolerance to colchicine. No patient experienced real toxicity.
Patient quality of life and self-reported outcome	Statement 7: Active disease and intolerance to colchicine affect quality of life.	Overall, 20.1% of patients experienced fatigue or chronic pain, 26.6% experienced limitations in daily activities, and 19.6% lost school/work days.

A recent multicentre and longitudinal study provided the possibility to verify in real life the actual impact of these statements concerning colchicine management (Table 5). In this study, 221 (125 children, 96 adults) Italian FMF patients treated with colchicine were followed for a median follow-up of 3.7 years [128]. Compliance to the drug was generally high (Figure 4). A complete response (absence of any fever episodes and per-

sistent normalisation of inflammatory parameters) was achieved in 55.2% of patients. As expected, 7.7% of patients were classified as resistant (≥one episode/month) according to the EULAR recommendations [128]. However, almost 30% of the patients were classified as partial responders since they presented a significant reduction in the number of fever episodes/year with fewer than one episode per month. Out of the partial responders, around 70% of them displayed a few episodes per year (from one to four); however, a relevant percentage (≈30%) displayed a rather high number of episodes per year (from five to eight) (Figure 5). Interestingly, in all age groups, a relevant proportion (almost 20%) of patients with residual disease activity were still on their colchicine starting dose (Figure 6). The maximal recommended colchicine dose (1 to 3 mg/day according to the age group) was not achieved in any of the resistant patients or patients with an incomplete response. This study provides evidence for the general treatment with colchicine in real life [128].

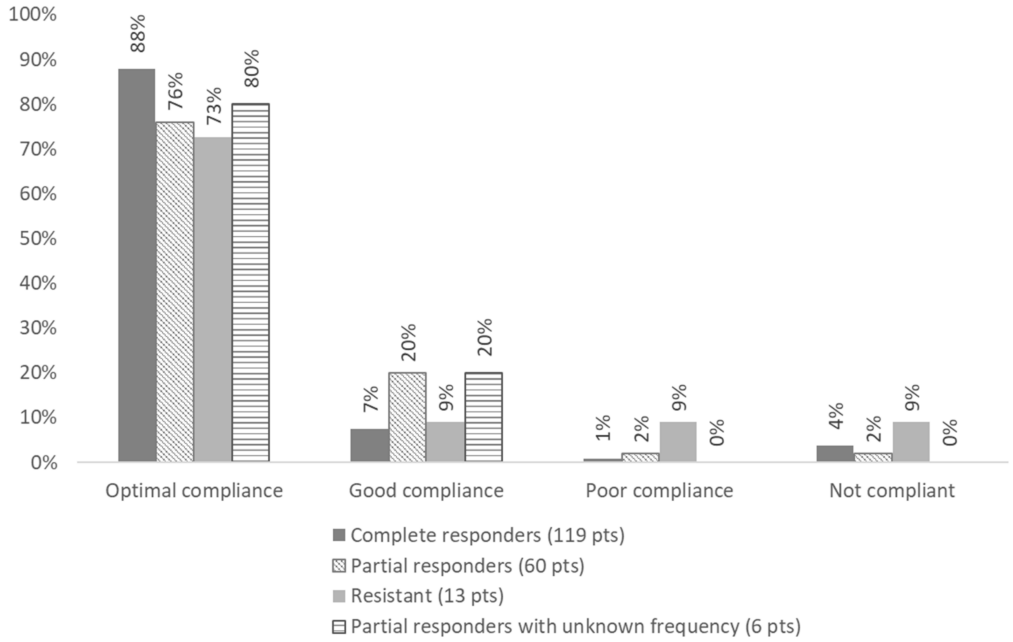

Figure 4. Compliance with colchicine treatment according to disease activity. Optimal compliance (compliant with >90% of prescriptions). Good compliance (compliant with 50–89% of prescriptions). Poor compliance (compliant with less than 50% of prescriptions) [125].

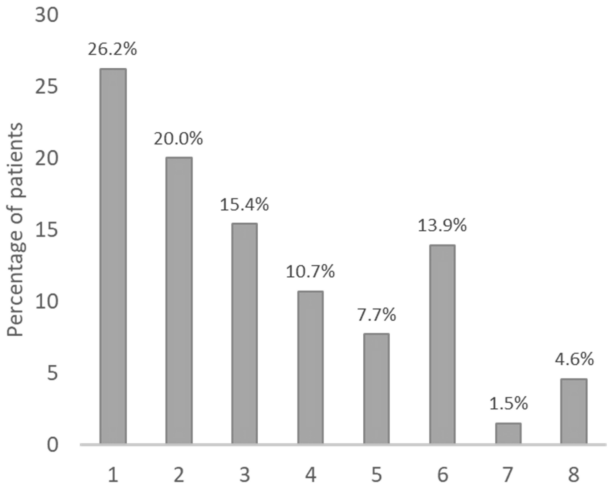

Figure 5. Number of episodes/year in patients with incomplete response to colchicine and less than one episode/month (partial responders) [128].

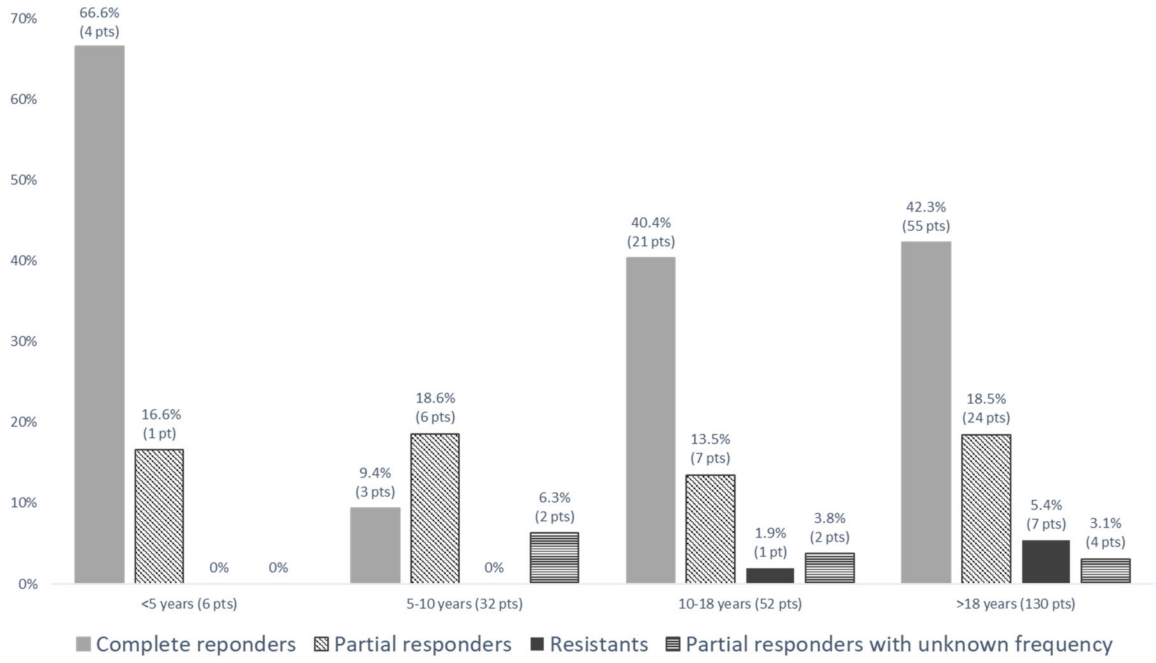

Figure 6. Colchicine response by age group in patients still receiving equal or less than the starting dose [128].

On the other hand, almost 30% of patients with a partial response, who were not considered resistant according to the current EULAR recommendations, reported a limitation in at least one item related to their quality of life, with a limitation in daily activities/presence at school or work or the presence of chronic pain or fatigue [128].

11. Interlukin-1 Inhibitors

Given that pyrin is implicated in the synthesis of IL-1, which is probably the strongest inducer of inflammation, its inhibition represents a new approach to treat FMF.

Three different types of anti-IL-1 treatments are available. Anakinra is a human recombinant non-glycosylated analogue of the IL-1 receptor antagonist (IL-1Ra) [129]. Rilonacept is a fusion protein engineered to contain the extracellular domain of type I IL-1 receptor fused with the Fc portion of IgG1. Canakinumab is a fully humanised monoclonal antibody of the class IgG1 that acts specifically against IL-1 beta [130].

11.1. Anakinra

Being an analogue of the receptor IL-1Ra, anakinra can competitively inhibit the binding of both IL-1α and β; however, there is no significant difference in the biological activities of either cytokine. It is administered as a daily subcutaneous injection [131].

Over the last several years, evidence for the important role of anakinra in the prevention of serositis attacks in patients with colchicine-resistant FMF has emerged. Anakinra was the drug that showed a higher degree of efficacy in colchicine-resistant patients in one of the first reports from the Eurofever registry [132]. The first RCT on the efficacy of anti-IL1 treatments was conducted in 2017, showing the efficacy and safety of anakinra for the treatment of colchicine-resistant FMF compared to a placebo [133]. The mean and SD were 1.7 $\pm$ 1.7 attacks per patient per month in the anakinra group versus 3.5 $\pm$ 1.9 attacks in the placebo group (P $\pm$ 0.037). However, considering site-specific attacks, the difference between the anakinra and placebo groups reached significance only for attacks in the joints. In this respect, anakinra may be complementary to colchicine, which often fails to prevent attacks in the joints while suppressing activity in other sites. There were no severe adverse events over a 20-month follow-up period.