UNIVERSITA' DEGLI STUDI DI GENOVA

DOTTORATO DI SCIENZE PEDIATRICHE XXXVI CICLO

DISEASE COURSE IN FMF PATIENTS AT THE TIME OF BIOLOGICS: FIRST REPORT FROM THE LONGITUDINAL EUROFEVER COHORT STUDY

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Anno accademico 2023-2024

I. Introduction

1. The historical background

Familial Mediterranean Fever (FMF) is the oldest and the most frequent autoinflammatory disease. It is a hereditary periodic fever syndrome characterized by self-limited episodes of fever and polyserositis; renal amyloidosis is the main long-term complication. FMF was particularly frequent in the populations originating from the Mediterranean basin, such as Turks, Armenians, Jews and Arabs (1). The MEFV gene (from MEditerranean FeVer), located on the short arm of chromosome 16, was described for the first time in 1997 by International and the French Consortium (2). The protein encoded by the MEFV gene was initially called "Marenostrin" in reference to the Latin name of the Mediterranean Sea. Alternatively, the name "Pyrin" was given by the International consortium to recall the Greek name of fever.

Before the identification of the causative gene, the description of the disease can be traced back to the ancient history of Mediterranean populations, being the Galen among the first to report it almost two thousand years ago. This condition continued to be part of the Mediterranean history throughout centuries despite migrations and merging of different cultures and people. In 1945, Siegal defined "benign paroxysmal peritonitis" as an under-diagnosed and "unusual clinical syndrome" in himself and other patients: "*The characteristics of this disorder are constant and distinctive. The syndrome is characterized by recurrent paroxysms of severe abdominal pain with fever which may be as high as 105° F [=~41.6°C]. Chilliness or a shaking chill may accompany the attacks. Involvement of the peritoneum is indicated by the subjective symptom of marked abdominal soreness and the objective finding of widespread, exquisite direct and rebound tenderness*" (3). The name Familiar Mediterranean Fever was given in 1955 by professor Heller, and his study group, (4) and became universal.

In 1972, the first anecdotal observations on the efficacy of colchicine were provided by Goldfinger (5). This event represented a revolution for management of FMF, decreasing the frequency and intensity of the attacks, and preventing renal amyloidosis, the most worrisome complication of uncontrolled FMF. Amyloid A (AA) amyloidosis results from continuous

inflammation and unrestrained secretion of acute phase reactants. Colchicine was able to reduce the incidence of amyloidosis by reducing the levels of sub-clinical inflammation (6).

2. Epidemiology

FMF is prevalent in the countries surrounding the Mediterranean Sea, especially affecting Turks, Arabs, Armenians and Non-Ashkenazi Jews. Turkey is probably the country with the greatest prevalence, which is reported to be 1:1000 overall, with interregional differences. A nationwide multicentre study done in Turkey (7) , shows that patients with FMF originate mainly from the non-Mediterranean regions, with over 70% of the cases from central and eastern Anatolia and inner Black Sea regions. Additional studies have revealed further differences in distribution, with the north-western region of Turkey having a prevalence as low as 6:10000 (8). Similarly, in Italy, the distribution of cases varies between Northern and Southern districts, the latter having a much higher occurrence of FMF. This phenomenon may be explained, at least partially, by the ancient colonization of the area by Greeks and Arabs and by the Jews migratory fluxes (9).

It is possible that MEFV mutations arose in pre-Biblical times and Jews, being genetically isolated, might represent the most likely candidate founder population of several common MEFV mutations (10), with a prevalence in Israel of roughly 1-2:1000. In the Armenian population, the same prevalence has been calculated (11). Additionally, the rate of carriers of FMF mutations in Armenians was shown to be 1:5, as high as in North African and Iraqi Jews, Turks, but lower than Moroccan Jews (1:3.5) and Muslim Arabs (1:4.3). Such an elevated number of carriers, resulting from a founder effect, does not correlate with the real prevalence of patients with a diagnosis of FMF, since the detection of a single mutation (heterozygosity) does not help in making the diagnosis (12).

It has also been hypothesized in the past that the high carrier rate of the MEFV gene mutations in certain populations is the result of an evolutionary advantage against tuberculosis (13) or brucellosis (14). The recent insights on the role of the Pyrin Inflammasome as crucial sensor against infection from microbes producing exotoxins outlined the possible selective advantage of MEFV carriers towards the infection of *Yersinia Pestis* during the different devastating plagues hitting the Mediterranean basin during the centuries (15).

In addition to the above countries, FMF is found in North African countries, Greece, Crete, France, Germany, and the US. In most of these countries, the presence of FMF is largely related

to robust emigration from the Mediterranean countries. Many studies have shown the presence of different severity of FMF according to the country of residence, totally or partially independent of the pathogenicity of MEFV variants and ethnicity. The incidence of amyloidosis is much higher in Turks and Armenian patients living in their country of origin in respect to the same population emigrated in northern Europe or the US (16). The same phenomenon was also reported in children by the international Eurofever registry that showed how children living in western European displayed a less severe disease activity independently from their ethnicity (17). These observations likely reflect the burden of environmental factors (i.e. infections) as possible triggers for a more robust inflammatory response in the Mediterranean countries.

Finally, a milder form of FMF is also present in Japan, with a lower prevalence of abdominal manifestations, a higher median age of onset and a lower frequency of complications (AA amyloidosis) as compared to Mediterranean patients, probably due to the differences in the MEFV gene mutations (18).

3. Pathogenesis

Pyrin, the protein product of the MEFV gene, is an immunoregulatory molecule made up of 781 amino acids, interacting with the inflammasome components that can be activated in response to microbes. The protein is mainly expressed in granulocytes and dendritic cells and within serosal and synovial fibroblasts (19).

Pyrin contains a N-terminal eponymous PYD domain, central B-box zinc finger, bZIP transcription factor and coiled-coil domains and a C-terminal B30.2 domain (Figure 1).

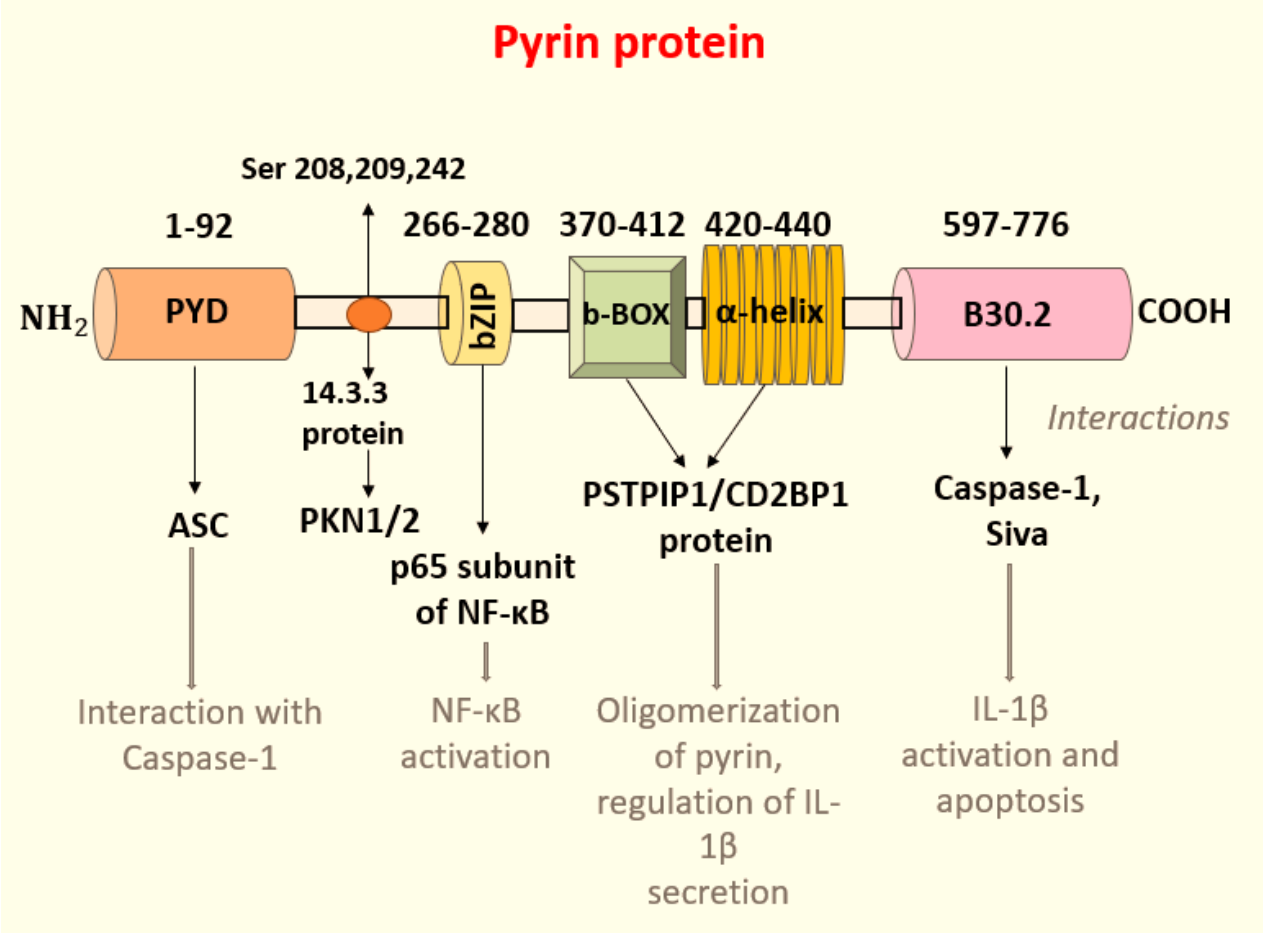


Figure 1 Schematic representation of Pyrin protein. Pyrin is an approximately 95 kDa protein made up of five domains: a PYD or PYRIN domain (1-92), bZIP transcription factor domain (266-280), B-box zinc finger (370-412), α helix coiled-coil domain (420-440) and a C-terminal B30.2 domain (597-776). N-terminal PYD domain is responsible for the interaction with ASC (apoptosis-associated speck-like protein with a caspase recruitment domain), which in turn mediates the CARD (caspase recruitment domain)- CARD homotypic interface with caspase-1. bZIP transcription factor basic domain promotes NF-kB activation via the interaction with its subunit p65. The box zinc finger and the α helix domain are involved in the oligomerization of pyrin and the regulation of IL-1β secretion. B30.2 domain harbors most of FMF-causing mutation being it functionally important in the activation of the pyrin inflammasome. B30.2 interacts with caspase-1 and pro-apoptotic protein Siva. Three residues of pyrin, serines 208, 209, and 242, are responsible for interacting with 14.3.3 regulatory molecule that participate in the phosphorylation via PKN 1/2 (serine/threonine protein kinase C-related kinase 1/2).

Most of FMF causative mutations are found in the B30.2 domain (20). The distinctive structure of PYD domain (amino acids 1–300), identified for the first time when MEFV gene was cloned, was not analogous to any other protein domain know at the time, hence it was named PYD or PYRIN domain. Since its discovery it has been found in more than 20 proteins regulating inflammation (21). It is responsible for the homotypic interaction with ASC, an apoptosis-associated speck-like protein that promotes the activation of caspase-1 (22). Typically, ASC oligomerizes with one of the NLRP proteins, and with procaspase-1 through homotypic CARD (Caspase recruitment domain) interactions to make up the inflammasome. This complex brings two molecules of precursors pro-caspase-1 into proximity, leading to autocatalysis and therefore

the release of the active catalytic p20 and p10 domains of caspase-1 (23). Caspase-1, in turn, cleaves the pro-form of IL-1β into its active form (Figure 2).

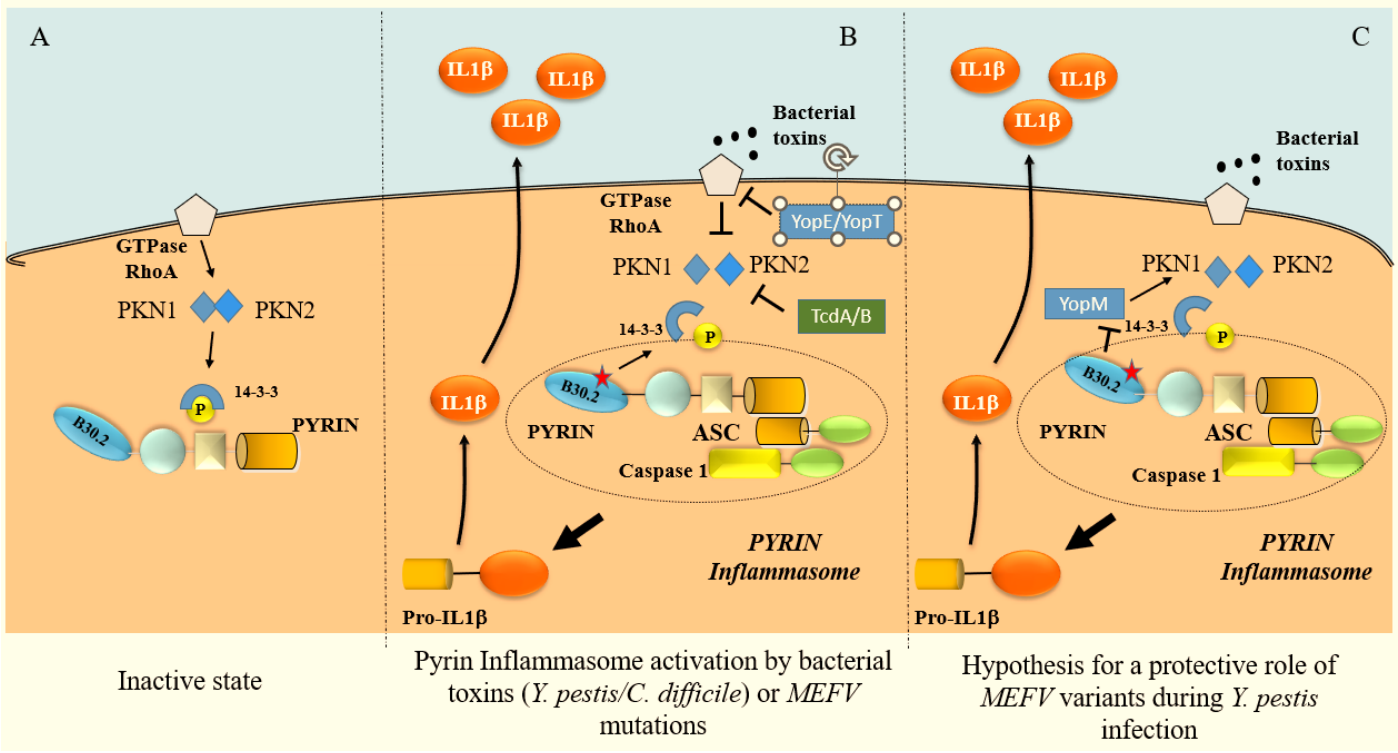


Figure 2 Mechanism of pyrin inflammasome. A. Phosphorylated pyrin in inactive state. In steady state condition, RhoA promotes inactive configuration of pyrin by inducing its phosphorylation, mediated by the serine-threonine kinases PKN1 and PKN2. B30.2 domain mutations are likely to control pyrin phosphorylation by inhibiting the binding of kinases to pyrin. B. Pyrin inflammasome assembly promoted by exotoxins or pathogenic MEVF mutations. Toxins produced by some bacteria (ie. YopE and YopT from *Yersinia Pestis*) directly inactivate RhoA; others toxins, such as TcdA/B from C. difficile, directly inactivate PKN1 and PKN2. The final result is the inhibition of PKN1 and PKN2 activation with the consequent de-phosphorylation of pyrin. Pathogenic MEFV mutations of exon 10 (B30.2 domain) interfere with the binding of 14-3-3 protein to Pyrin, leading the Pyrin protein more susceptible to de-phosphorylation. Dephosphorylated pyrin is active and able to interact with ASC and Caspase-1, forming the pyrin-inflammasome. IL-1β is cleaved to its active form as a result of the autocatalysis and activation of two precursors molecules of caspase-1. C. Hypothesis on the possible protective role of MEFV mutation during *Yersinia pestis* infection. The Y.pestis-induced virulence factor YopM directly inhibits pyrin inflammasome formation by promoting PNK1/2-mediated pyrin phosphorylation. MEFV mutations on B30.2 domain attenuate the Pyrin-YopM interaction, thus interfering with the YopM anti-inflammatory activity.

In order to better characterize FMF, researchers started to investigate the biological function of unmutated pyrin. In the past, depending on the experimental settings, pyrin was shown to both activate and inhibit the caspase-1/IL-1β signalling pathway (24–26). The process of pyrin-inflammasome inhibition has been described to depend on RhoA phosphorylation (20). RhoA activates the serine-threonine kinases PKN1 and PKN2 that phosphorylate pyrin. Phosphorylated pyrin binds to regulatory proteins, such as 14-3-3, that avoid the formation of the pyrin inflammasome (Figure 2A). The toxins produced by bacteria are able to inactivate

RhoA and, in turn, inhibit PKN1 and PKN2 activation, with the consequent de-phosphorylation of pyrin (Figure 2B). Pyrin is, therefore able to interact with ASC and Caspase-1, forming the pyrin-inflammasome, with the consequent cleavage and secretion of IL-1 (Figure 2B). FMF-associated mutations of the B30.2 domain make the protein less prone to phosphorylation, thus leading to constitutive activation of the pyrin inflammasome, influencing the interaction of the regulatory protein 14-3-3 with Pyrin (20) (Figure 2B).

Due to the autosomal recessive mode of transmission, FMF was believed to be caused by a loss-of-function mutation of pyrin. However, pyrin knockout mice develop normally and do not exhibit an inflammatory phenotype. A further challenge to the loss-of-function theory is given by the fact that some individuals display the disease despite being heterozygous for one single mutation (27,28). Moreover, asymptomatic carriers of MEFV mutations can have elevated acute-phase reactants (29). Homozygous Pyrin "knockin" harboring mutant human B30.2 domains, but not pyrin-deficient, mice exhibited spontaneous inflammation similar to but more severe than human FMF (30). Caspase-1 was constitutively activated in knockin macrophages and active IL-1β was secreted after LPS stimulation, as observed in FMF patients. The inflammatory phenotype of knockin mice was reversed by crossing with IL-1 receptor-deficient or adaptor molecule ASC-deficient mice, but not NLRP3-deficient mice. These pivotal studies provide the final evidence for an ASC-dependent NLRP3-independent inflammasome in which gain-of-function pyrin mutations cause FMF (30).

The clinical consequence of the dosage effect of MEFV variants was described in children with periodic fevers in which the prevalence of FMF-related clinical manifestations was significantly correlated to the number and pathogenicity of the MEFV variants carried by the patients (31). Similarly, the degree of over secretion of IL-1 from FMF monocytes after LPS stimulation is proportional to the number and pathogenicity of MEFV variants carried by the patients (32).

Dependence from RhoA makes pyrin-inflammasome distinct from other inflammasomes which are activated through pattern-recognition receptors: it does not directly interact with PAMPs and DAMPs. Instead, it indirectly senses and responds to pathogen virulence factors that modify RhoA, acting as a molecular "guard", that senses alteration in the homeostasis of the cell (33).

The crucial role of the Pyrin Inflammasome in the response to pathogens inducing toxins release (such as *Y. pestis*) led to the fascinating hypothesis of a possible selective advantage for individual carriers of MEFV causative during plague times (34) (Figure 2C). within fact the *Yersinia pestis* virulence factor called YopM stimulates the PKN-1/2-mediated phosphorylation

of pyrin and thereby the inhibition of pyrin inflammasome reducing IL-beta secretion in response to the infection (35). In turn, MEFV pathogenic variants attenuate the Pyrin-YopM interaction, thus interfering with the YopM-induced interleukin1β suppression (Figure 2C). Leukocytes from FMF patients release heightened IL-1β specifically in response to *Y. pestis,* as compared to healthy controls. *Y. pestis*-infected knock-in mice for pathogenic MEFV variants exhibit IL-1-dependent increased survival relative to wild-type knock-in mice. Thus, MEFV pathogenic mutations confer heightened resistance to *Y. pestis* (35).

3.2 IL-1β

Interleukin-1 (IL-1) is one of the most potent pro-inflammatory cytokines. Two distinct ligands (IL-1α and IL-1β) bind the IL-1 type 1 receptor (IL-1R1) inducing a pro-inflammatory cascade leading to the production of mediators, such as prostaglandins, cytokines, and chemokines (36). The IL-1α precursor is constitutively present in most epithelial cells and is fully active. On the contrary, IL-1β is synthesized as an inactive precursor, only after the activation of the cells, typically after the stimulation of toll-like receptors. The activation of IL-1α is contingent on proteolytic cleavage by caspase-1 (37). IL-1 is able to induce the myeloid differentiation primary response gene 88(MyD88), and therefore translocation of active NF-κB to the nucleus. NF-κB-dependent genes, such as NLRP3, pro-IL-1β, pro-IL-18, and IL-6, are the mediators in this process. The central role of IL-1 in the innate inflammatory processes, and therefore in autoinflammatory diseases, explains the clinical success of IL-1 blocking agents in treating such conditions (38,39).

4. Genetics & Genotype-phenotype correlation

MEFV gene is made up of 10 exons and is localized on chromosome 16p13.3. To date, more than 300 variants have been identified and reported in the INFEVERS database (Infevers, Sarrauste de Menthiere et al., http://fmf.igh.cnrs.fr/ISSAID/infevers/index.php) (figure 3), however their relative frequencies and pathogenicity are not known for most of them. The hot-spots of the FMF-causing MEFV variants were found on exon 2, at position 148, and on exon 10, at position 680 and 694 (40). In Turkey the most frequent mutations are M694V, E148Q, M680I and V726A (41); whereas in the Israeli community the common mutation for non-Ashkenazi Jews is M694V (76.8%) (42) and E148Q for Ashkenazi Jews (43). Whilst V726A is the most encountered mutation among Arabs. These last three variants represent probably the most ancient MEFV mutations, and it is calculated that their appearance in the Middle East

(Mesopotamia) could be dated more than 2,500 years ago (44). The hypothesis is that migrations of few families around the Mediterranean basin during the centuries lead to a founder effect. Further evidence of this phenomenon comes from study of an isolated Jew community in Palma de Mallorca, the "Chuetas", formed by 18 families in whom more than 60 FMF patients have been diagnosed and their genotypes overlapped with those observed in North African Jews (45).

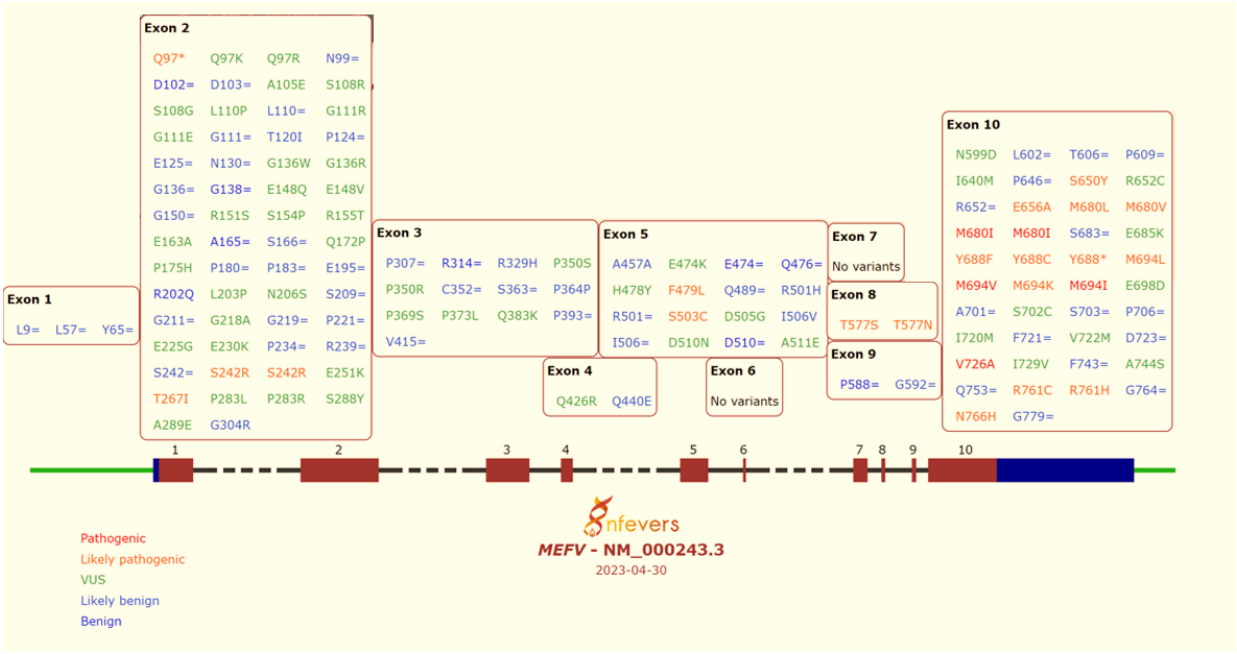


Figure 3 MEFV mutational spectrum based on free source INFEVER online database. In red and orange are annotated, respectively, pathogenic and likely pathogenic mutations; in green are marked VUS (variance of unknown significance); while, in light blue and blue are reported likely benign and benign variants. Hot spots for pathogenic mutations are found on exon 2 and 10.

The modification of the methionine residue at position 694 was described as a high penetrance mutation since the discovery of the Pyrin gene in 1997. In addition, despite the disease being typically considered recessive, some patients with classical FMF phenotype were reported to have a seemingly dominant transmission (46).

Indeed, as far as phenotype-genotype correlations is concerned, several observations showed that a more severe phenotype, with high fever, splenomegaly and musculoskeletal manifestations is usually associated with high penetrance mutations (47,48), such as M694V, which also seems to confer a less favourable response to colchicine (49). On the other hand, the low penetrance variant E148Q has been suggested to have an aggravating effect: dominant transmission when allelic to M694I with a second wild type allele, amyloidosis when allelic to V726A with a second mutated allele (50). Mild phenotype or incomplete penetrance have also been described in patients with K695R or P369S (40).

In the past, twin studies proved full concordance between monozygotic twins and a 30% concordance rate between dizygotic twins, with some degree of clinical variability (51). However, in addition to the MEFV gene, some other genetic loci may impact on the pathogenesis of the disease as modifiers, such as MICA (major histocompatibility complex class I chain–related gene A) (52), and polymorphisms of the SAA1 (Serum amyloid A1) gene (53).

5. Clinical features

The clinical presentation of FMF can be variable, likely depending on its genetic heterogeneity and environmental factors. However, the clinical picture is usually very suggestive of the underling disease. It is typically characterized by recurrent episodes of fever and systemic inflammation with (pleural and abdominal) serositis and arthritis. Patients present since childhood short-lasting self-resolving attacks of fever, abdominal, chest or joint pain with systemic inflammation (54). Periodicity is not strict, and episodes may occur from once a week up to one every three-four month or more in untreated patients. These events are usually very disabling and in clear contrast with complete well-being in the attack free periods (55).

Several triggers have been identified for the attacks, such as stressful events (56), cold exposure (57) and menstrual cycle in pubertal and post-pubertal females (58). A prodrome was found to be a common manifestation of FMF, experienced by about 50% of the patients. Most commonly, it entails a sensation of general malaise and discomfort, neurological manifestations such as headache (59) or abdominal pain.

In 90% of cases, the disease onset is in childhood; 65% below 10 years (60). A young age of onset (<2 years) is associated with a more severe course of illness, higher penetrance mutations (61) and a more pronounced delay in diagnosis (62). Fewer studies have been made on adult-onset FMF, but it has been reported to have a mean age of clinical onset of 32.5 years, milder symptoms and lesser or no disease complications (63). For these reasons adults suspected to have FMF, may also benefit from different diagnostic tools as compared to the paediatric populations (64).

Disease flares in FMF are typically associated with elevation in acute phase response (C-reactive protein, CRP and serum amyloid A, SAA). Sometimes high CRP and SAA levels are also found during the attack-free periods, indicating a still active disease and a strong risk factor for the development of amyloidosis due to chronic inflammation (65). Another recently

described, yet not characterized, player in the development of proteinuria and amyloidosis in FMF, are high serum levels of galectin-3 (gal-3). Gal-3 is a b-galactoside-binding lectin highly expressed on innate immune cells and involved in pro-inflammatory and pro-fibrotic pathways (66). Galectin-3 could be used in the future as a prognostic biomarker for the development of renal damage in FMF and other conditions (67).

3.3 Fever

Fever is present in 96% of inflammatory episodes, ranging from 38° to 40°C (68) . It appears suddenly and lasts from 12 to 72 hours. The typical cycle displays a spontaneous and fast rising in the temperature followed by a plateau and rapid decrease. In young children, fever may represent the unique disease manifestation at onset, with the subsequent development of a typical clinical presentation (including serositis) over the next 2.9 ± 2.2 years (61).

3.4 Abdominal manifestations

Abdominal pain is extremely frequent during fever episodes, being reported in 94% of patients (7) and is secondary to a sterile inflammation of the peritoneum. Usually, the pain is severe and induces the patient into an antalgic position and bed rest. Sometimes it may mimic an acute abdomen with rebound tenderness, reduced peristalsis, distension and rigidity of abdominal muscles. Radiographic features may reveal small air-fluid levels and can wrongly guide caregivers toward an explorative laparoscopy and possibly unnecessary appendicectomy or cholecystectomy. Indeed, appendectomy in FMF patients was found to be much higher than the reported rate in the general population (40% vs. 12-25%); whilst the number of non-inflamed appendectomies was much more (80% vs. 20%) (69). Most of the time, peritonitis is completely resolved within 2–3 days without sequelae. Constipation is often observed during the episode, while diarrhoea occurs in 10–20% of episodes. In addition, the described vomiting rate is of approximately 30% in children (31). Possible long-term complications consequent of repeated bouts of peritoneal inflammation are abdominal adhesions, leading to sub-occlusion and infertility; that were frequently observed in the pre-colchicine era (70).

Palpable enlarged spleen is found according to different series variably in 10-60% of patients (31,55). Splenomegaly not related to amyloidosis can be detected in ultrasonography and is usually the direct result of bowel sterile inflammation, which also causes abdominal micro-lymphadenopathy (71).

3.5 Pleurisy and Pericarditis

Pleural serositis is also for chest pain, which manifests with dyspnea and bilateral respiratory and pleural auscultatory friction sounds in the involved site of the pleura (72). The frequency changes in diverse study groups and ranges between 20-60%. An additional X-ray finding is transient small pleural effusion which resolves within 48 hours after the episode (60).

Pericarditis is rare but more frequent than in the general population (around 7 per 1000 cases vs 0.5) (73) and may present with retrosternal pain and ST abnormalities on electrocardiogram. It usually occurs years after the diagnosis, even though uniquely, it could be its first sign (74). Interestingly, idiopathic recurrent acute pericarditis (IRAP) can be seen in both autoinflammatory (such as FMF and TRAPS) and autoimmune conditions (systemic lupus erythematosus, rheumatoid arthritis, progressive systemic sclerosis and others) suggesting a mixed pathogenesis, involving both innate and adaptive immune systems (75).

3.6 Musculoskeletal symptoms

Joint manifestations are observed in 50% of cases and manifest as transient arthralgia or mono/oligoarthritis. Recurrent monoarthritis is the most common presentation, and it usually affects knee, hip and/or ankle (76). The arthritis is typically associated with a robust inflammatory reaction with redness and swelling of the involved joint, arthrocentesis results in an aseptic exudate with high amount of inflammatory cells. Synovitis usually resolves after 24-48 hours with the same evolution of abdominal or chest attacks with no sequelae. However, progressively destructive arthritis has been described in the literature in some cases (77). A less common manifestation of joint involvement in FMF is spondyloarthropathy, which is often HLA–B27 negative.

Myalgia may be associated with FMF, ranging from spontaneous generalized self-resolving muscle ache, exertional leg pain and, less commonly, protracted febrile myalgia. Exertional leg pain has been characterized as less intense post-exercise acidification of muscle of FMF patients as compared to controls (78). Protracted febrile myalgia syndrome (PFMS) is a rare complication of FMF which cannot be prevented with colchicine. PFMS is a long-lasting (4-6 weeks), intense and disabling muscle pain, usually in the lower limbs, associated to high grade fever, high inflammatory parameters, but normal muscle enzymes and non-specific inflammatory changes in the EMG. A high signal intensity distributed around myofascicles in the inflamed muscles can be detected by MRI (79). It requires high dosage cortisone treatment or anti-Il1 agents (80).

3.7 Other manifestations

As for other serosal membranes, inflammation of the tunica vaginalis producing orchitis may be another event occurring in FMF patients. The frequency of acute scrotum has been reported up to 9% in some studies (81). Scrotal attacks are unilateral, self-limited, painful with red swelling of the testicle, lasting 48-72 hours. 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 [79]), 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, ankle joint and dorsum of foot. Foot erythema is usually associated with ankle arthritis. It may be triggered by physical effort and subside spontaneously within 48 to 72 hours of rest. Recurrent oral ulceration was found to be relatively frequent (10%) in FMF (82). Interestingly, heterozygous mutations in exon 2 of the phosphorylation site of Pyrin (c.726C > G; p.Ser242Arg) have been associated to a unique phenotype distinct from the typical FMF, characterized by severe acne and pyoderma gangrensum, in the so-called Pyrin-associated autoinflammatory diseases with neutrophilic dermatosis (PAAND) (33).

3.8 Associated diseases

Several inflammatory and autoimmune conditions have been associated to FMF and MEFV gene mutations, probably due to common dysregulations of 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) (83). Demyelinating central nervous system disease multiple sclerosis (MS) is enriched in FMF patients compared to Israeli and Turk populations (84,85). 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 were found to be increased in a big cohort of patients with FMF when compared with those in the literature (86). Finally, a strong association with hidradenitis was also shown (87).

6. Diagnostic and Classification Criteria

The first set of diagnostic criteria was created for adults by the experts in Tel Hashomer Hospital (60) (Table 1), and thirty years later, they were refined by *Livneh et al*. (88) (Table 2).

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

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

However, a diagnostic standard with high specificity and sensitivity was necessary also 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 (89) (Table 3). These were validated in Turkish children cohort, 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 of duration of attacks, diagnosis prior to appendicectomy etc.).

Table 2. Livneh criteria
(at least 1 major criteria, or 2 minor criteria, or 1 minor criterion plus at least 5 supportive criteria, or 1 minor criterion plus at least 4 of the "first" five supportive criteria.)

Major criteria
Typical attack* of generalized 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 abdomen
Incomplete attack* involving 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
Nonproductive laparotomy with removal of a "white" appendix
Consanguinity of parents

** Typical attacks are defined as recurrent (≥3 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 1 or 2 features, as follows: 1) normal temperature or lower than 38°C; 2) attacks longer than 1 week or shorter than 6 hours, 3) no signs of peritonitis recorded during acute abdominal complaint, 4) The abdominal attacks are localized, 5) The arthritis involves joints other than those specified.*

Table 3. Yalcinkaya-Ozen (childhood) criteria
(at least 2 out of 5 criteria)

Fever (axillary temperature >38°C, 6-72 hours of duration, ≥3 attacks)
Abdominal pain (6-72 hours of duration, ≥3 attacks)
Chest pain (6-72 hours of duration, ≥3 attacks)
Oligoarthritis (6-72 hours of duration, ≥3 attacks)
Family history of FMF

The pediatric criteria yielded a better sensitivity but a poorer specificity than in previous criteria when applied to an international cohort of children from either European or Eastern Mediterranean regions (90). Conversely, Tel Hashomer criteria displayed the best specificity

but a poor sensitivity. A higher specificity was meant to minimize the diagnostic failure or delay, although FMF diagnosis still needed to be refined with the inclusion of genetic data (91).

Indeed in 2015, a group of experts built up a set of evidence-based recommendations through a systematic literature review (92). 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 by genetic testing (Strength B). This statement is currently matter of debate, since many authors believe that the term FMF should be applied only to patients carrying MEFV mutations (Ben-Chetrit), FMF being a genetic condition.

In this line, the new evidence based Eurofever/PRINTO classification criteria, developed for inherited recurrent fevers in 2019, included for the first time an association of clinical and genetic variables, resulting in a high sensitivity and high specificity classification tool (93) (Table 4). These criteria are able to differentiate among different inflammatory conditions and despite being classification criteria, mainly aimed to research purposes (clinical trials, translational studies), they may provide both clinical based- and genetic-based guides useful for the diagnostic orientation in FMF in clinical practice.

Table 4. Eurofever/PRINTO classification criteria for FMF

Presence of confirmatory MEFV genotype* and at least **one,** or not confirmatory MEFV genotype** and at least **two,** among the following:
Duration of episodes 1–3 days.
Arthritis.
Chest pain.
Abdominal pain
A patient with (1) evidence of elevation of acute phase reactants (ESR or CRP or SAA) in correspondence to the 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 trans compound heterozygous for one pathogenic MEFV variants and one VUS, or biallelic VUS, or heterozygous for one pathogenic MEFV variant.*

7. 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 the molecular analysis, a committee has developed guidelines classifying genes as: a) clearly or likely pathogenic; b) variants of unknown significance or VOUS; c) clearly or likely benign (94).

M694V is considered a very severe mutation, and if present in homozygosity, even asymptomatic individuals should be considered for treatment (Strength A, (92)). M694V, V726A, M694I, M680I and E148Q account for 70–80% of the cases in Mediterranean countries (95). However, in case an uncommon variant is identified, physicians and molecular geneticists can utilize the INFEVER database. INFEVERS (Internet Fevers), created in 2002, is an online database for the documentation of all information available on mutations in autoinflammatory disorders-related genes (http://fmf.igh.cnrs.fr/ISSAID/infevers/index.php) (96). It was conceived as a universal tool to gather and share all data on the mutational spectrum of HRF genes in a centralized location to highlight information that can be missed if reported separately. More specifically, to overcome the challenges of interpreting VOUS with low frequency which may function as susceptibility alleles to inflammation or new and rare genetic variants associated with a clear phenotype (97). To date, on this website are documented more than 350 MEFV variants together with the classification status (benign, likely benign, VOUS, likely pathogenic, pathogenic) and the centre that made the notification (Figure 3). The information provided by INFEVER can be complemented by other databases on 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).

8. Treatment

3.9 Colchicine

i. Colchicine: mechanism of action

Colchicine is the oldest known drug still used today (98) . It is an alkaloid derivative of the plant *colchicum autumnale.*

In the 60s, colchicine' ability to bind microtubules was discovered, revealing its antimitotic action (99). However, colchicine action in FMF still remains to be elucidated to some degree. Microtubules, molecular targets of colchicine, operate pleiotropically within the cell governing intracellular organelle and vesicle transport, secretion of cytokines and chemokines, cellular migration and division, and regulating gene expression. 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 microtubules' properties (100). Colchicine action leads to an impaired neutrophil chemotaxis by diminished expression of L-selectin on neutrophils cell membranes, and of E-selectin on endothelial cells [100] and neutrophil function by inhibiting superoxide production [101]. Moreover, colchicine dampens the activation of macrophages and the degranulation of mast cells [102] and interferes with TNF-α pro-inflammatory actions [103] and thereby with NF-kB signalling cascade (101–106).

In addition to the indirect action on chemotaxis, motility and stimulation of leukocytes, colchicine has been demonstrated to inhibit NLRP3 inflammasome, thereby suppressing caspase-1 activation in gout. 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, colchicine by acting on microtubes is thought to activate - or release from inhibition – RhoA, resulting in suppression by phosphorylation of the pyrin inflammasome assembly.

ii. 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). Altered expression of this transporter protein may signify sub-optimal therapeutic effects or drug toxicity. Colchicine is eliminated by biliary excretion and through the stool (107,108). A significant role in colchicine metabolism is played by enteric and hepatic cytochrome P450 3A4 (CYP450 3A4), which catalyses demethylation of colchicine to the inactive metabolites, 2- and 3-demethtylcolchicine. This is relevant in that drugs modifying the activity of this cytochrome can result in colchicine-induced toxicity. Finally, 20% of drug disposal is accounted 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 dosage. 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. Blood dyscrasias and neuropathies are features of chronic type overdose (109).

High colchicine concentrations are extremely toxic, leading to a severe microtubules disarrangement. The affected cells experience a halt in protein assembly, endocytosis, exocytosis, cellular motility, mitosis, cardiac myocyte conduction and contractility [110]. The

accumulation of these mechanisms may lead to a multi-organ dysfunction and failure, consisting in three overlapping phases. Around 10-24 hours from ingestion severe gastrointestinal manifestations appear. From 24 hours 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. If the patient survives, recovery occurs in a week or so (110,111).

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 (111).

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

Finally, in addition to abdominal side effects of colchicine, evidence points toward the exitance of a drug-induced lactose intolerance in treated FMF patients, which can be corrected with lactose-free diet (112).

The risk of colchicine-driven oligo-/azoospermia is still matter of debate. Probably the frequency of azoospermia is influenced more by the underlying pathology, or by the presence of testicular amyloidosis, rather than the drug itself. Indeed, healthy volunteers do not experience infertility under colchicine treatment and colchicine does not cause reduced sperm motility (113–116).

For what concerns female fertility, colchicine therapy throughout pregnancy seems to carry no substantial teratogenic or mutagenic risk when used at recommended doses [117]. Additionally, colchicine was shown not to be associated with a higher rate of miscarriage, stillbirth, low birth weight nor early delivery (117,118).

iii. Colchicine: management in Familial Mediterranean fever

Early independent RCTs demonstrated that daily colchicine is highly effective in preventing attacks in this disorder in a dose-related fashion (5,6,119).

According to the ongoing EULAR recommendations [120], the "starting dose" of colchicine was defined as ≤0.5 mg per day in children with less than 5 years, 0.5 mg per day in 5-10 years-old children and 1 mg in those aged 10-18 years and in 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 step-wise fashion until the maximal tolerated dose. The maximal dose is considered to be 1 mg/day in children aged less than 5 years, 2 mg/day in pre-pubertal children and 3 mg/day in post pubertal children and adults (120,121).

Very few studies on colchicine dose per-kilo have been completed. In a cohort of children, the mean effective colchicine dosage was calculated to be 1.46±0.41 mg/m^2/day or 0.05±0.02 mg/kg/day in children <5 years; 1.19±0.3 mg/m^2/day or 0.03±0.01 mg/kg/day in children 6-11 years old; 0.84±0.2 mg/m^2/day or 0.027±0.01 mg/kg/day in children 11-15 years old (122) (mean dose of the whole group was 1.16±0.45 mg/m^2/day or 0.03±0.02 mg/kg/day). These findings are coherent 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 the best correlation of colchicine intake is with the body surface area (~1.03 mg/m^2/day) (123).

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

1. Colchicine resistance

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

In 2016, The European League Against Rheumatism (EULAR) in its recommendations for management of FMF, defined resistance as 1 or more attacks per month in compliant patients who had been receiving the maximally tolerated dose for at least 6 months. More recently, a consensus of experts updated these recommendations through several statements, including some recommendations on adherence, dose adjustment criteria and quality of life (124). The conclusions of the Consensus were schematically reported in seven statements (Table 5).

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 (125). Compliance to the drug was generally high (Figure 4). A complete response (absence of any fever episodes and persistent normalization off inflammatory parameters) was achieved in 55.2% of patients. As expected, 7.7% of patients were classified as resistant (≥ 1 episode/month), according to the EULAR recommendations. However, almost 30% of the patients were classified as partial responders since they presented a significant reduction of the number of fever episodes/year with less than 1 episodes per month. Out of the partial responders, around 70% of them displayed few episodes per year (from 1 to 4), however, a relevant percentage (≈30%) displayed a rather high number of episodes per year (from 5 to 8) (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). In none of the patients resistant or with an incomplete response, the maximal recommended colchicine dose (1 to 3 mg/day according to the age group) was achieved. This study provides evidence of a general treatment with colchicine in the real life.

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

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

	Delphi expert consensus statements (Özen et al, 2021 [124])	**Results of national multicenter 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 with their prescribed colchicine treatment.	83.8% displayed an optimal adherence (> 90% of prescription); 10.6% a good adherence (50-89% of prescriptions); 2.0% poor adherence (<50% of prescriptions); 3.5% 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	Patients taking colchicine dose without adjustments were: 71.3% of patients < 5 years; 35.3% of those 5-10 years;

	adjustment of maximal dose in children depending on age (and weight).	57.0% aged 10-18 years; 67.1% of adults.
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 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 persistence of fever attacks, despite optimal treatment. 54.2% patients had a complete disease control; 30.1% patients had < 1 episode/month for 3 months; 8.5% had ≥1 episode/month for 3 month and 7.2% had persisting disease with 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.	20.1% of patients experienced fatigue or chronic pain, 26.6% limitations in daily activities, and 19.6% have lost school/work days.

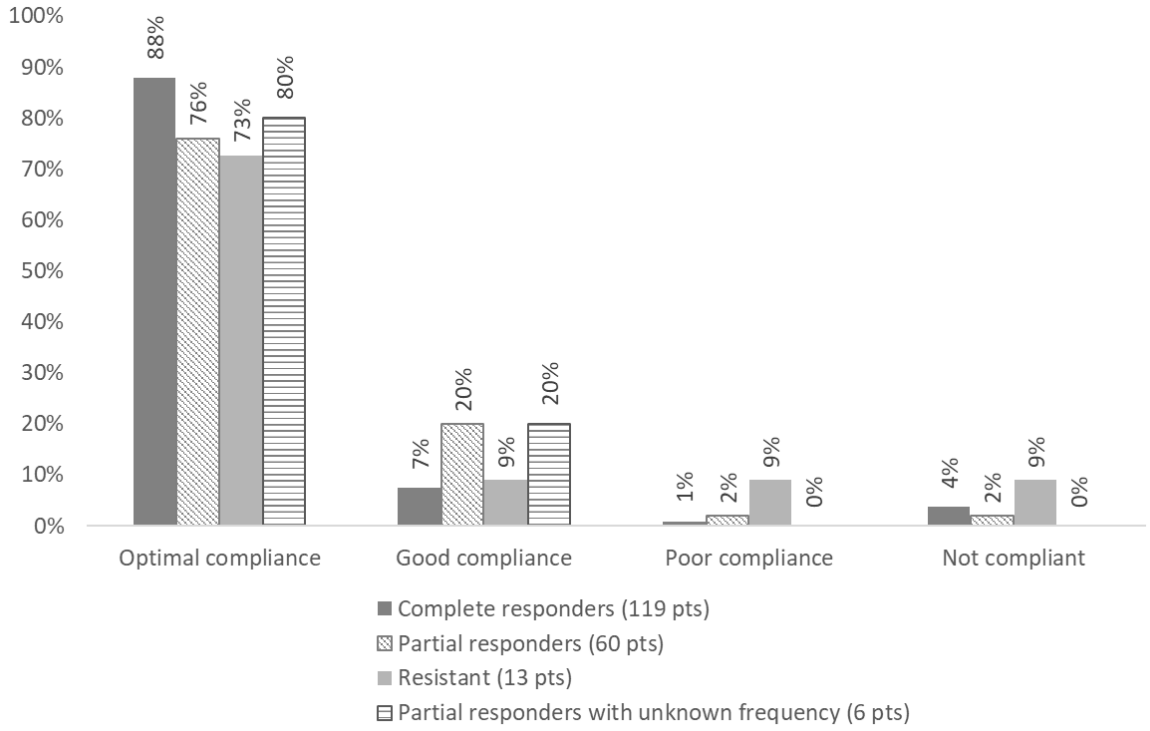


Figure 4 Compliance to colchicine treatment according to disease activity. Optimal compliance (compliant to >90% of prescriptions); Good compliance (compliant to 50%-89% of prescriptions); poor compliance (compliant to less than 50% of prescriptions) (125)

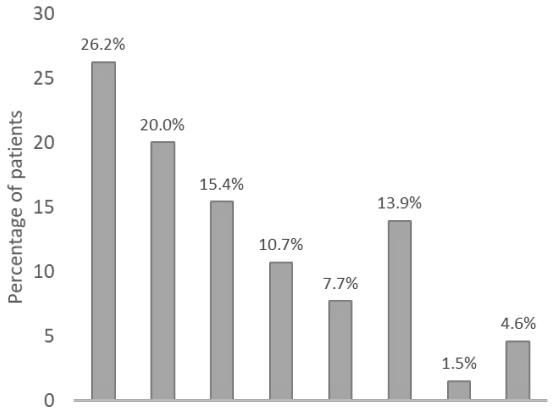


Figure 5 - Amount of episodes/year in patients with incomplete response to colchicine and less than 1 episode/month (partial responders).

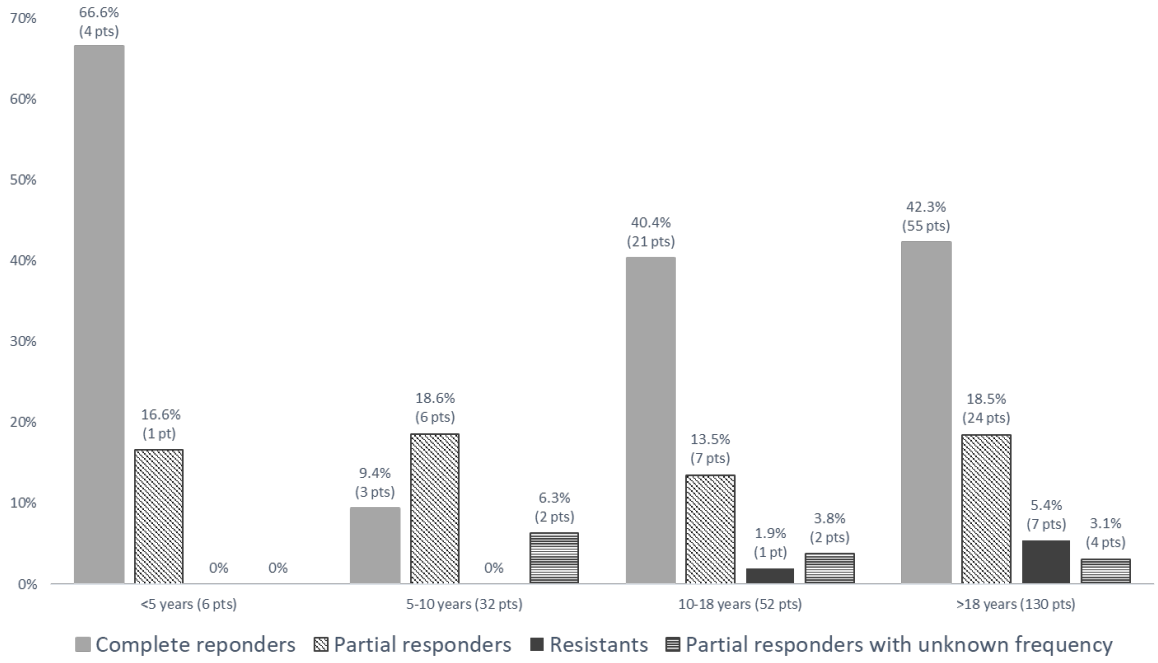


Figure 6 - Colchicine response by age group in patients still receiving equal or less than starting dose (125)

3.10 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 un-glycosylated analogue of the IL-1 receptor antagonist (IL-1Ra) (126). 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 humanized monoclonal antibody of the class IgG1 that acts specifically against IL-1 beta (127).

iv. Anakinra

Being an analogue of the receptor IL-1Ra, anakinra can competitively inhibit binding of both IL-1α and β, however here is no significant difference between the biological activities of either cytokine. It is administered as a daily subcutaneous injection (128).

Over the last several years, evidence of an important role for anakinra in the prevention of serositis attacks in patients with colchicine-resistant FMF has emerged. Anakinra was the drug showing the higher degree of efficacy in colchicine-resistant patients in one of the first report from the Eurofever registry (129). The first RCT on the efficacy of an anti-IL1 treatments was

conducted in 2017, showing the efficacy and safety of anakinra for the treatment of colchicine-resistant FMF compared to placebo (130). The mean was SD 1.7 ±1.7 attacks per patient per month in the anakinra group versus 3.5 ± 1.9 attacks in the placebo group (P ± 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, that often fails to prevent attacks in the joints while suppressing activity in other sites. There were no severe adverse events over a 20-month period follow-up.

On the other hand, several case studies reported improvement in renal function in patients with amyloidosis following anakinra treatment (126,131). The use of anakinra during pregnancy resulted to be safe (132,133) and is currently recommended in colchicine-resistant women.

The most common side effect is injection site reaction [134]. Albeit uncomfortable, these usually resolve within 2–3 weeks of treatment initiation, however they may be so severe to prompt patient to interrupt treatment (134,135).

As for other anti-cytokine treatments major concern is the risk of infection. Nevertheless, in comparison to other biologic agents, anakinra has an unparalleled safety benefit deriving from short half-life and effect duration and has demonstrated a remarkable record of safety (136).

v. Rilonacept

Rilonacept is a very high affinity “cytokine trap” consisting of fusions between the constant region of IgG and the extracellular domains of two distinct cytokine receptor components involved in binding the cytokine. It is administered weekly through an injection.

The first randomized placebo-controlled study in FMF with an anti IL-1 agent was performed with rilonacept. The study included 12 patients and rilonacept significantly reduced the number of FMF attacks and had an acceptable safety profile, with no serious side effects associated with this drug (137,138).

vi. Canakinumab

Canakinumab is the only FDA-approved cytokine blocker for the treatment of colchicine-resistant FMF in the United States (139). Its long half-life allows a monthly subcutaneous administration.

The first report in the literature of the successful administration on canakinumab in a patient with FMF and chronic arthritis after failing anakinra, etanercept and low dose prednisone, and methotrexate was published in 2011. A significant decrease in proteinuria in the amyloidosis-complicated FMF patients was observed. All the series reported that patients benefit from canakinumab (140–143,143)and others, also in terms of quality of life (65).

The efficacy of the treatment was confirmed when randomized against a placebo in a cohort of colchicine-resistant FMF patients together with TRAPS and mevalonate kinase deficiency (MKD) patients (144). A complete response occurred in 71% of FMF patients when treated with canakinumab (150 or 300mg subcutaneously every 4 weeks). Patients who did not have a complete response had a lower number of days of fever per year. When an extended dosing regimen (canakinumab every 8 weeks) was evaluated, absence of flares was maintained in approximately half the patients with colchicine-resistant FMF. In this study, no deaths, opportunistic infections, or cancers were reported.

In all three cohorts, infections were more numerous in the canakinumab group than in the placebo group, serious infections being rare (7.4 per 100 patient-years). Three patients had to discontinue treatment because of neutropenia. The long-term efficacy and safety of Canakinumab in the phase 3 cluster trial of the same study was recently reported (145).

3.11 Anti IL-6 drugs

IL-6 is elevated in the serum of FMF patients during attacks, and its potentiality as a biomarker to distinguish between acute phase and remission (146) and drug target was investigated. Tocilizumab (TCZ) is a humanized monoclonal anti IL-6 receptor antibody, binding to soluble and membrane receptors and down regulating IL-6 synthesis, and as a consequence possibly suppressing SAA production. Indeed, the result from a series of 12 patients with AA amyloidosis secondary to FMF treated with TCZ, showed an improvement in attacks.

Long term safety of TCZ is now being investigated in a Japanese multicentre placebo-controlled, randomized, double-blind trial on colchicine-resistant or colchicine-intolerant FMF (147).

9. Conclusive remarks and future perspective

In conclusion, Familial Mediterranean Fever (FMF) is the first inflammatory condition for which a causative gene was identified and represents a prototype of a monogenic autoinflammatory disease condition. In recent years, significant progress has been made in understanding the pathogenic mechanisms related to this condition. Early diagnosis and prompt treatment with colchicine can effectively manage symptoms and prevent complications.

Future research efforts should focus on developing more effective therapies for FMF patients who are unresponsive to colchicine. Further studies are also needed to identify new genetic mutations that contribute to FMF and to explore the possible association between FMF and other diseases. Moreover, the development of biomarkers for monitoring disease progression and response to therapy would be beneficial in improving the clinical management of FMF. In addition, genetic counseling and family screening programs should be implemented to identify asymptomatic carriers and prevent the transmission of the disease to future generations. In summary, while significant progress has been made in understanding and treating FMF, there is still much to be done to improve patient outcomes and quality of life. With continued research efforts and collaboration among healthcare professionals, we can work towards better management and ultimately a cure for this condition.

II. Objective

The aim of this study was to describe the clinical features and disease outcome of a multicentre longitudinal cohort of FMF patients and to analyze their response to treatment, safety, quality of life and compliance information.

III. Methods

Data were extracted from the Eurofever registry and collected through a secured platform hosted on the Paediatric Rheumatology International Trials Organisation (PRINTO) website (http://www.printo.it). Fifteen countries were involved in the study, having their patients included in the registry. The study was approved by the Gaslini ethical review board. Informed consent was obtained from each patient before enrolment. The registry was anonymous, and patients were identified by a private alphanumeric code.

All patients with a clinical and/or genetic diagnosis of FMF according to the participating centre were included in the study. The cross-sectional version of the registry exists since 2009, the longitudinal version is available since 2014. Additional questions on quality of life and compliance have been collected in the registry since 2022. The dataset was closed in November 2023. All centres were asked to update the clinical information of the patients until the last follow-up. Baseline information, clinical manifestations, molecular diagnosis and response to treatment were analysed.

Baseline data included: age, gender, country of birth, country of residence, ethnicity, consanguinity, age at the time of disease onset and diagnosis, and date of first and last visit to the referring centre. Besides, molecular diagnosis was integrated with information on the mutations found (according to the InFever database, http://fmf.igh.cnrs.fr/ISSAID/infevers), the type of the molecular analysis performed (point mutations, more informative exons, or complete gene sequencing) and the laboratory having carried out the tests.

Clinical characteristics encompassed all the manifestations from disease onset to diagnosis and proper treatment. The items examined were: (i) nature of the fever episodes (duration,

frequency, regular frequency pattern, etc); (ii) presence and frequency (always or often/sometimes) of the symptoms and (iii) presence of triggers.

Treatment and dosage were also registered, provided that for each patient anthropometric values (height and weight) were added. Additionally, persistence of symptoms and their frequency, was monitored longitudinally in the follow up visits.

Response to treatment was defined as *complete* (absence of clinical manifestations and normal laboratory parameters), or *incomplete* (persistence of fever episodes and/or some elevation of acute phase reactants). According to the number of fever episodes the *incomplete responders* were further classified as: ii) patients that experienced more than 1 episode per month (120) ii) patients experienced less than 1 episodes per month; iii) patients that experienced some disease activity but with unknown frequency of residual episodes for those patients presenting fever episodes in which the precise frequency was not referred by the centre.

According to the ongoing recommendations, the "starting dose" of colchicine was defined as ≤0.5 mg/die in children with less than 5 years, 0.5 mg/die in 5-10 years-old children and 1 mg in those aged 10-18 years and in adults. Maximal dose was defined as 1 mg/day in children aged less than 5 years, 2 mg/day in pre-pubertal children and 3 mg/day in post pubertal children and adults. Frequencies and percentages were used as descriptive statistics for categorical variables. To describe numerical variables median and range were used.

The compliance was classified as i) optimal if the patient was compliant to more than 90% of prescriptions, ii) good if the patient was compliant to 50-90% of prescriptions, iii) poor if the patient was compliant to less than 50% of prescriptions and iv) not compliant at all.

Quality of life was assessed through a self-reported or family reported questionnaire with a visual assessment score (VAS). The questionnaire investigated the level of perceived activity of the disease, the level of tiredness, the number of days out of school or work, and the subjective feeling due to the illness. The questionnaire distributed to the patients and family is detailed in Table 6. In addition, a VAS score with the valuation of disease activity by the physician was recorded.

STATA software was used for statistical analysis. General statistics are reported as frequencies and percentages for categorical variables and median and range for numerical variables. All descriptive results are given with 95% confidence intervals (95% CIs). Analyses involved the

chi-square test for categorical variables and t-test for continuous variables. $P < 0.05$ was considered statistically significant.

Table 6. Quality-of-life assessment questionnaire
Considering all the symptoms, please evaluate the level of activity of your illness in the last month
In everyday life activities during the last month, you got tired very quickly
Considering all the ways the illness affects your life, please evaluate how you have felt in the last month
Number of days out of school or work in the last month due to your illness

IV. Results

In November 2023, 1104 patients with FMF were enrolled in Eurofever registry by 15 countries (Table 7, Figure 7), most patients were enrolled by European or Middle East centers (Table 8, Figure 8). Complete clinical, genetical data and information about treatment received at baseline were available of the entire cohort. Longitudinal follow-up visits were available for 497 patients.

Table 7. Country of enrollment*	
Italy	425 (38%)
Turkey	102 (9.3%)
Greece	96 (8.6%)
Netherlands	79 (7.2%)
Armenia	72 (6.4%)
Slovakia	67 (6.1%)
Egypt	64 (5.8%)
Spain	64 (5.8%)
France	56 (5.1%)
Germany	41 (3.7%)
Austria	27 (2.5%)
Argentina	3 (0.3%)
Czech Republic	5 (0.5%)
Denmark	2 (0.2%)
Ukraine	(<0.1%)

***N (%)**

Figure 7 Country of enrollment of the baseline cohort

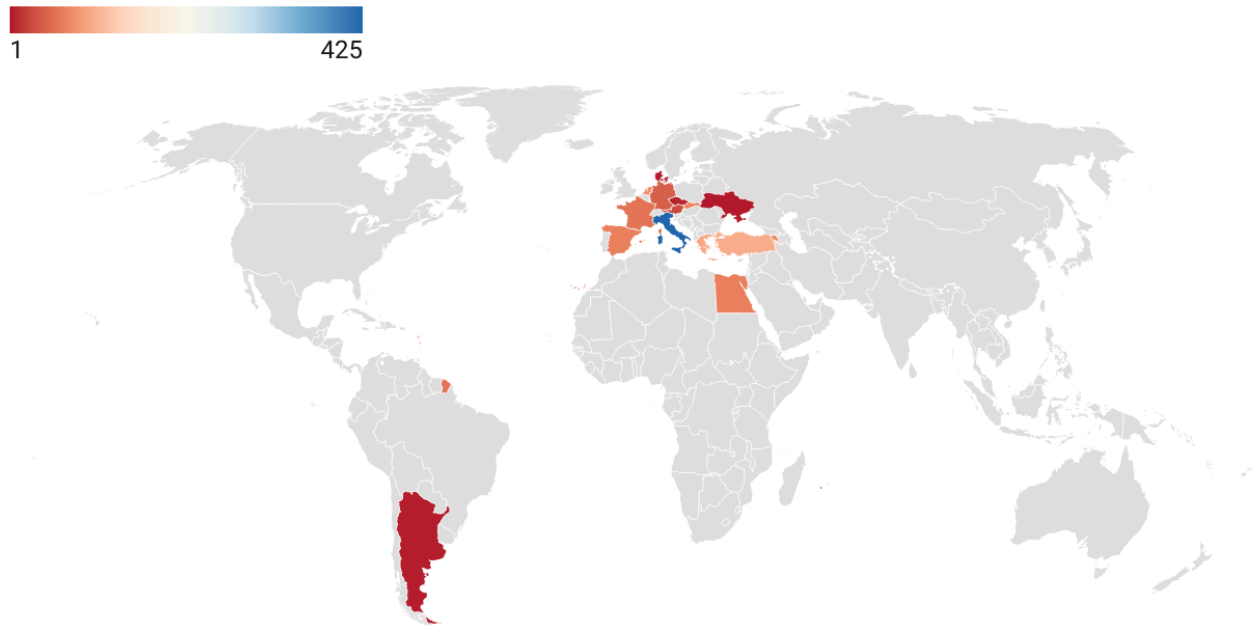
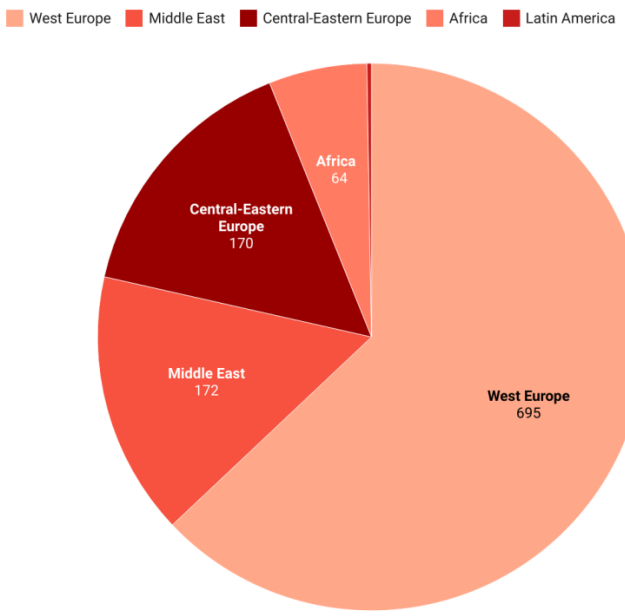


Table 8. Geographic area of Eurofever centers*	
West Europe	695 (62.9%)
Middle East	172 (15.6%)
Central-Eastern Europe	170 (15.4%)
Africa	64 (5.8%)
Latin America	3 (0.3%)

* N(%)

Figure 8 Geographic area of the enrolling centers.



10. Description of the baseline cohort

3.12 Demographic characteristics of the baseline cohort

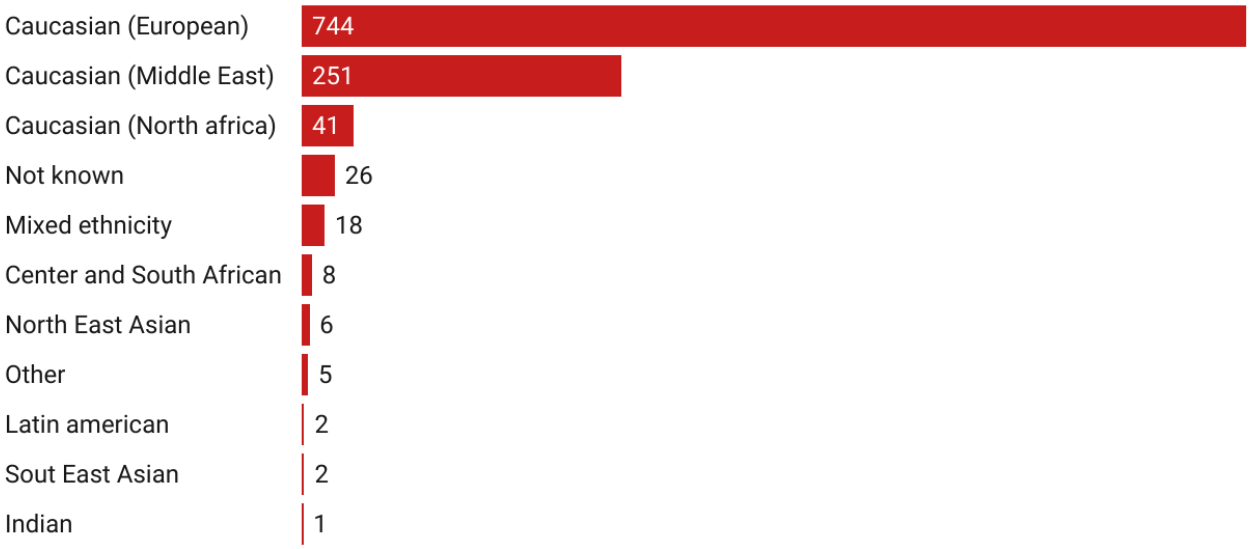
We had complete baseline information of 1104 patients, 574 were males and 530 females, 845 patients were child at time of enrolment (Table 9). The median age at disease onset was 3.8 (5-95th centile 0.4 - 28.1); the median diagnostic delay was 2.4 years (0.1 – 28.7); the median age at enrolment was 7.9 (1.7 – 50.8).

Table 9. Demographics characteristics	
N	1104
Gender (M : F)	574:530
Pediatric patients, N (%)	845 (77.0%)
Adult patients, N (%)	259 (23%)
Age at onset, median years (range)	3.8 (0.4 - 28.1)
Age at diagnosis, median (range)	7.4 (1.9 – 47.5)
Age at enrollment, median (range)	7.9 (1.7 – 50.8)
Diagnostic delay, median years (range)	2.4 (0.1 – 28.7)

Most patients were Caucasian as shown in Table 10 and Figure 9, the European Caucasian was the most frequent ethnicity in the cohort.

Table 10. Ethnicity	**N**	**%**
Caucasian (European)	744	67,4
Caucasian (Middle East)	251	22,7
Caucasian (North Africa)	41	3,7
Not known	26	2,4
Mixed ethnicity	18	1,6
Center and South African	8	0,7
North East Asian	6	0,5
Other	5	0,5
Hispanic (for US)	2	0,2
Sout East Asian	2	0,2
Indian	1	0,1

Figure 9 Ethnicity in the baseline cohort



3.13 Genotype in the baseline cohort

Complete information about genotype were available for 1061 patients. 34 patients never received any genetic test, in 9 patients the molecular analysis was done but the results were not available (probably the test was still ongoing). The type of genetic test performed is listed in the Table 11: most patients received a sanger of the most relevant exons or point mutations, while NGS and whole exome sequencing were performed in less than 8% of patients.

Table 11. Genetic test method performed

	N	%
Most relevant exons	467	44.0
Most relevant point mutations	202	19.0
Complete gene screening	184	17.3
Not known	126	11.9
NGS	72	6.8
Whole exome sequencing	10	0.9

101 patients were homozygous for M694V mutations, while other 424 (40.0%) carried at least one M694V mutations, alone or in compound heterozygosity with another pathogenic or VUS variant (Table 12, Figure 10). The complete list of MEFV variants reported in the cohort is shown in Table 13.

Table 12. Presence of M694V variant in the cohort		
	N	%
M694V homozygous	101	9.5 %
M694V heterozygous or compound heterozygous	424	40.0 %
M694V negative	536	50.5 %

Figure 10 Presence of M694V variant in the baseline cohort

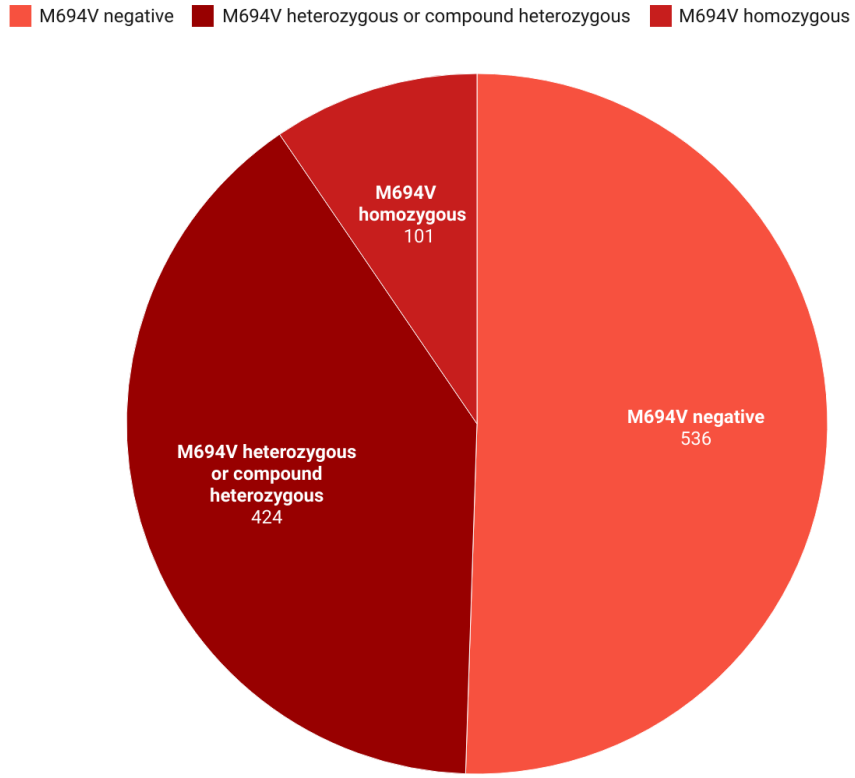


Table 13. MEFV variants in the entire cohort					
MEFV variants	**N**	**%**	**MEFV variants**	**N**	**%**
M694V/WT	234	21,2	M694I/M680I	2	0,2
M694V/M694V	101	9,1	M694I/R761H	2	0,2
M694V/M680I	48	4,3	M694V/E167D	2	0,2
E148Q/WT	45	4,1	M694V/I591T	2	0,2
WT/WT	44	4,0	V726A/T267I	2	0,2
M680I/WT	43	3,9	-330G>A/WT	1	0,1
M694V/V726A	41	3,7	A744S/I640M	1	0,1
K695R/WT	28	2,5	A744S/P369S	1	0,1
M694I/WT	28	2,5	A744S/R202Q	1	0,1
V726A/WT	28	2,5	E148D/WT	1	0,1
M694V/E148Q	27	2,4	E148Q/R202Q	1	0,1

Table 13. MEFV variants in the entire cohort

MEFV variants	N	%	MEFV variants	N	%
A744S/WT	24	2,2	E148Q/R348H	1	0,1
M680I/M680I	24	2,2	E148Q/R408Q	1	0,1
M680I/V726A	23	2,1	E148V/P369S	1	0,1
M694V/R202Q	18	1,6	E163A/P369S	1	0,1
P369S/R408Q	18	1,6	E230K/R42W	1	0,1
M694V/M694I	15	1,4	G219G/WT	1	0,1
M694I/E148Q	13	1,2	G304R/WT	1	0,1
R202Q/WT	13	1,2	I591T/E148Q	1	0,1
I591T/WT	12	1,1	I591T/Q440E	1	0,1
M694V/R761H	11	1,0	I591T/R348H	1	0,1
P369S/WT	11	1,0	I692DEL/E148Q	1	0,1
M694V/K695R	10	0,9	I692DEL/I692DEL	1	0,1
E148Q/P369S	9	0,8	I720M/WT	1	0,1
M680I/R761H	9	0,8	K477N/WT	1	0,1
M694I/V726A	9	0,8	K695R/K695R	1	0,1
R761H/E148Q	9	0,8	K695R/P369S	1	0,1
R202Q/R202Q	7	0,6	M582L/WT	1	0,1
R761H/WT	7	0,6	M680I/A744S	1	0,1
V726A/E148Q	7	0,6	M680I/M680V	1	0,1
M694I/M694I	6	0,5	M694DEL/R202Q	1	0,1
M694V/E230K	6	0,5	M694I/I692DEL	1	0,1
V726A/S108R	6	0,5	M694L/WT	1	0,1
E148Q/E148Q	5	0,5	M694V/A289V	1	0,1
M680I/E148Q	5	0,5	M694V/A761H	1	0,1
V726A/M680I	5	0,5	M694V/E225G	1	0,1
F479L/WT	4	0,4	M694V/M680L	1	0,1
M694V/A744S	4	0,4	M694V/M680V	1	0,1
V726A/E167D	4	0,4	M694V/P369S	1	0,1
V726A/V726A	4	0,4	R202Q/A268V	1	0,1
A744S/A744S	3	0,3	R202Q/R348H	1	0,1
K695R/R202Q	3	0,3	R408Q/E148Q	1	0,1
R408Q/P369S	3	0,3	R408Q/WT	1	0,1
R761H/R761H	3	0,3	R653H/WT	1	0,1
S339F/WT	3	0,3	R717L/WT	1	0,1
V726A/F479L	3	0,3	R761C/WT	1	0,1
A289V/R202Q	2	0,2	T267I/WT	1	0,1
A289V/WT	2	0,2	V726A/E148D	1	0,1
E148Q/L110P	2	0,2	V726A/I591T	1	0,1
E167D/F479L	2	0,2	V726A/I692DEL	1	0,1
F479L/F479L	2	0,2	V726A/M693I	1	0,1
I640M/R653H	2	0,2	V726A/N270D	1	0,1
K695R/R42W	2	0,2	V726A/Q476Q	1	0,1
M680I/M694I	2	0,2	V726A/R761H	1	0,1
M694I/A744S	2	0,2	Y688X/E148Q	1	0,1

We applied the Infever classification of FMF genetic variant and the classified the genotype of the patients as a) confirmatory with biallelic pathogenic variants if the patient was homozygous or in trans (or biallelic) compound heterozygous for two pathogenic or likely pathogenic variants b) non confirmatory, but with monoallelic pathogenic variants, if the patient was heterozygous for one pathogenic MEFV variant and one VUS, or biallelic VUS, or heterozygous for one pathogenic MEFV variant, c) not informative or negative if the patients was wild type or heterozygous for one VUS variant or carried only benign or likely benign variants.

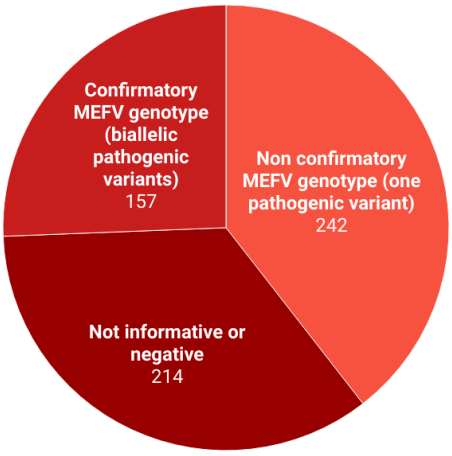
According to this classification we found that in our cohort most of patients carried at least one pathogenic variants and one third of them carried two biallelic pathogenic variants, 20.2% had were negative or with non-informative genotype (Table 14, Figure 11).

Table 14. Genotype classification according to Infevers in the baseline cohort

	N	%
Confirmatory *MEFV* genotype*	328	30.9 %
Non confirmatory *MEFV* genotype**	519	48.9 %
Negative or not informative genotype	214	20.2 %

* biallelic pathogenic variants; *monoallelic pathogenic variants

Figure 11 Genotype classification according Infever in the baseline cohort



Analyzing demographic characteristics according to genotype we observed that patients homozygous for M694V had a significantly lower age of onset compared to patients heterozygous for M694V or negative for that variant (Table 15).

Table 15. Age of onset according to M694V variant in the baseline cohort

		N	Mean	SD	p
Age at onset	M694V heterozygous or compound	424	7.06	9.39	<0.001
	M694V homozygous	101	4.42	5.29	
	M694V negative	536	8.08	9.43	
Age at diagnosis	M694V heterozygous or compound	424	13.40	15.24	0.002
	M694V homozygous	101	9.92	10.51	
	M694V negative	536	14.22	14.46	
Age at first visit	M694V heterozygous or compound	424	14.50	16.34	0.201
	M694V homozygous	101	12.07	14.18	
	M694V negative	536	14.87	15.44	

3.14 Clinical characteristics in the baseline cohort

Characteristics of episodes at baseline are detailed in Table 16. Median number of episodes per year was 12 (5-95th centile 2-25), median duration of episodes was 3 days (1-7) and 50% of patients reported a regular pattern of episodes. Table 5 report signs and symptoms associated with fever episodes and detail if a symptom was experienced at every episode or in an inconstant manner.

As expected, the most frequently reported symptom associated with fever was abdominal pain (81.3%), arthralgia (60.1%), fatigue (46.2%), myalgia (43.0%), malaise (39.6%) and chest pain (35.7%). Amyloidosis was reported in 13 patients at baseline visit, 1.2% of the cohort.

Table 16. Characteristics of episodes in the entire cohort			
General characteristics of episodes			
Episode per year*	12.0 (2.0 - 25.0)		
Duration of episode (days)*	3.0 (1.0 - 7.0)		
Regular pattern frequency of fever episodes°	511 (50%)		
Signs and symptoms during episodes			
	Often°	**Always°**	**Total°**
Fever	-	-	994 (90.4%)
High fever (>38°C)	426 (38.6%)	516 (46.7%)	942 (85.3%)
Low fever	324 (29.3%)	63 (5.7%)	387 (35.1%)
Abdominal pain	415 (37.6%)	483 (43.8%)	898 (81.3%)
Arthralgia	486 (44.0%)	177 (16.0%)	663 (60.1%)
Fatigue	330 (29.9%)	180 (16.3%)	510 (46.2%)
Myalgia	369 (33.4%)	106 (9.6%)	475 (43.0%)
Malaise	274 (24.8 %)	163 (14.8%)	437 (39.6%)
Chest pain	292 (26.4%)	102 (9.2%)	394 (35.7%)
Vomiting	270 (24.5%)	57 (5.2%)	327 (29.6%)
Diarrhea	249 (22.6%)	44 (3.9 %)	293 (26.5%)
Tonsillitis	34 (3.1%)	220 (19.9%)	254 (23.0%)
Arthritis	38 (3.4%)	186 (16.8%)	224 (20.3%)
Latero-cervical adenopathy	172 (15.6%)	35 (3.2%)	207 (18.8%)
Aftous lesions	167 (15.1%)	37 (3.4%)	204 (18.5%)
Headache	156 (14.1%)	27 (2.4%)	183 (16.6%)
Pleuritis	106 (9.6%)	12 (1.1 %)	118 (10.7%)
Painful lymph nodes	105 (9.5%)	9 (0.8%)	114 (10.3%)
Maculopapular rash	93 (8.4 %)	5 (0.5%)	98 (8.9%)
Peritonitis	68 (6.2%)	4 (0.4%)	72 (6.5%)
Erysipelas-like rash	61 (5.5%)	5 (0.5%)	66 (5.9%)
Generalized adenopathy	49 (4.4 %)	6 (0.5%)	55 (4.9%)
Urticarial rash	49 (4.4%)	5 (0.5%)	54 (4.9%)
Conjunctivitis	49 (4.4%)	4 (0.4 %)	53 (4.8%)
Pericarditis	43 (3.9%)	10 (0.9%)	53 (4.8%)
Splenomegaly	30 (2.7%)	16 (1.5%)	46 (4.2%)
Hepatomegaly	18 (1.6%)	6 (0.5%)	24 (2.2%)
Tenosynovitis	13 (1.2%)	4 (0.4%)	17 (1.5%)
Vertigo	12 (1.1%)	1 (0.1%)	13 (1.2%)
Amyloidosis	-	-	13 (1.2%)
*median (5-95th centile), °N (%)			

One fifth of patients reported triggers for fever episodes, such as stress, fatigue, infections, exercise, cold, menstruations, travel, vaccination, specific food and trauma (Table 17).

Table 17. Triggers reported for fever episodes	
Stress	77 (6.9%)
Fatigue	65 (5.9%)
Infection	54 (4.9%)
Exercise	45 (4.1%)
Cold	40 (3.6%)
Menstruations	32 (2.9%)
Travel	20 (1.8%)
Vaccination	12 (1.1%)
Food	12 (1.1%)
Trauma	8 (0.7%)

[1]n (%)

3.15 Concomitant diseases

221 patients have concomitant diseases (Table 18). As reported in previews studies most frequent concomitant diseases are gastroduodenitis, Juvenile Idiopathic Arthritis, spondylarthritis, vasculitis and inflammatory bowel syndrome. The most frequent vasculitis reported is Shonlein-Henoch purpura, the others were 2 cerebral vasculitis, one Kawasaki syndrome, one hemorragic vasculitis, one panarteritis nodosa, one urticarial vasculitis and one IgA nephropathy. Connectivits reported were one LES and one Sjogren syndrome. In the cohort 5 neoplastic disease were reported: one kidney carcinoma, one craniopharyngioma, one Wilms tumor and one pancreatic cancer.

Table 18. Concomitant diseases reported in the cohort*	
Gastroduodenitis (with or without H.pylori infection)	50 (4.5%)
Juvenile Idiopathic Arthritis	12 (1.1%)
Spondyloarthritis	10 (0.9%)
Inflammatory bowel syndrome	9 (0.8%)
Schonlein-Henoch purpura	9 (0.8%)
Other vasculitis	6 (0.5%)
Neoplastic disease	4 (0.4%)
Connectivitis	2 (0.2%)
Psoriasis	3 (0.3%)
Behcet-like syndrome	3 (0.3%)
Rheumatoid arthritis	2 (0.2%)

Hypogammaglobulinemia	2 (0.2%)
Celiac disease	2 (0.2%)
Fibromyalgia	2 (0.2%)
Autoimmune hepatitis	1 (0.1%)
CRMO	1 (0.1%)

*N(%)

3.16 Treatment in the baseline cohort

We analyzed the treatment received by the patients at baseline (Table 19).

873 patients (79.1%) received colchicine, which was the most frequent treatment, as expected. Around 12.9% of patients were off continuous therapy at baseline. 103 patients received anti-IL1, either anakinra or canakinumab, 55 anakinra and 75 canakinumab, out of them 31 received initially anakinra and then switched to canakinumab. 72 patients received anti-IL-1 in association with colchicine, while 31 patients received biologic treatment alone.

Table 19. Treatment at baseline

	Entire cohort *(1104 pts)*
Colchicine, *n (%)*	873 (79.1%)
Biologic treatment	103 (9.3%)
Anakinra, *n (%)*	55 (4.9%)
Canakinumab, *n (%)*	75 (6.8%)
Off therapy/other treatments, *n (%)*	143 (12.9%)
Duration of treatment	
Colchicine, *median years (range)*	3.8 (0.1 – 14.6)
Anakinra, *median years (range)*	1 (0.1 – 7.8)
Canakinumab, *median years (range)*	2 (0.1 – 7.6)

Forty patients were receiving other treatments as shown in Table 20: eleven methotrexate (MTX), twenty-five anti-TNF, seven sulfasalazine, seven tocilizumab, six azathioprine, one cyclosporine, one thalidomide. Colchicine was given concomitantly in 33 of them, three of them received anti-IL1 treatment exclusively while 5 were not receiving neither colchicine nor anti-IL1. Twelve patients have an associated disease (Juvenile Idiopathic Arthritis, spondyloarthropathy, vasculitis, Behçet disease, IBD).

Table 20. Other treatments	
Methotrexate	11
Adalimumab	9
Sulfasalazine	7
Tocilizumab	7
Azathioprine	6
Infliximab	6
Etanercept	6
Golimumab	4
Thalidomide	2
Ciclosporine	1

11. The longitudinal cohort

3.17 Demographic characteristics in the longitudinal cohort

Follow-up information was available in 497 patients, the percentage of pediatric patients at enrollment was 75% (Table 21). The median age at disease onset was 4.0 (5-95th centile 0.4-25.9); the median diagnostic delay was 2.9 (0.3 – 27.7); the median age at enrollment was 8.8 (2.1 – 50.9).

Table 21. Demographic characteristics of longitudinal cohort

N	497
Pediatric patients°	372 (74.8%)
Adult patients°	125 (25.2%)
Gender°	
Male	274 (55%)
Female	223 (45%)
Age at onset*	4.0 (0.4 - 25.9)
Age at diagnosis*	8.3 (2.2 - 44.9)
Age at enrollment*	8.8 (2.1 - 50.9)
Diagnostic delay (years)*	2.9 (0.3 - 27.7)

°N(%), *median (5-95th centile)

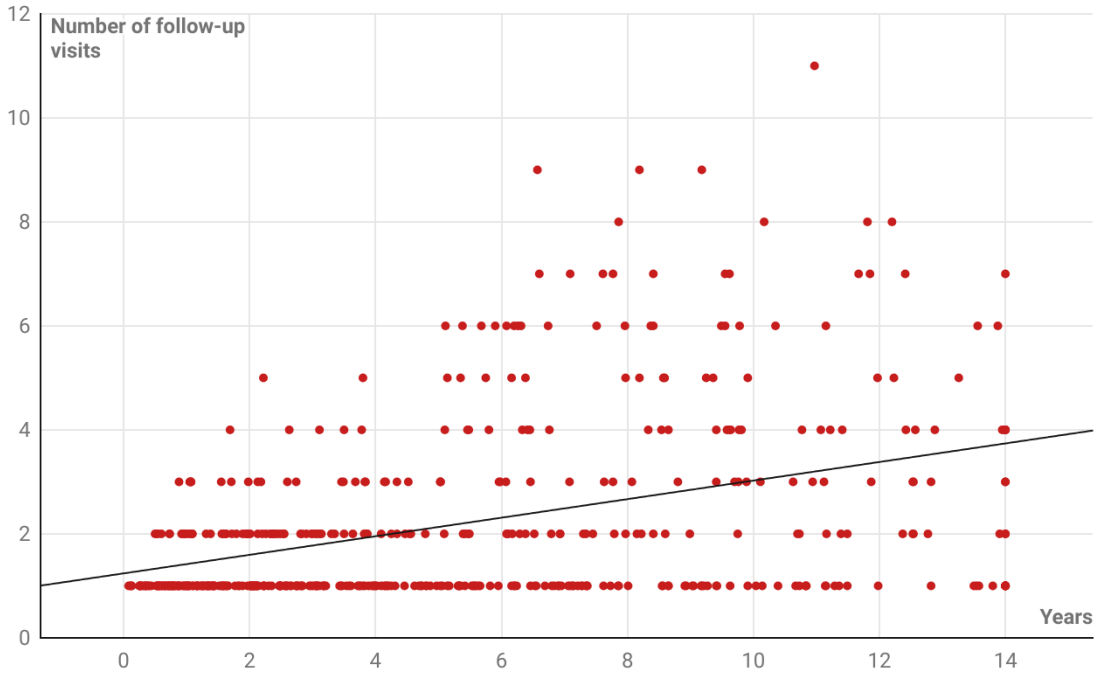
The mean number of follow-up visits was 2.2 (2-3), the median duration of follow-up was 4.3 years (0.6 – 12.8). The relationship between number follow-up visits and duration of follow-up is shown on Figure 12 and in Table 22.

Table 22. Duration of follow-up in years per number of follow-up visits

N of visits	Years of follow-up*	N of patients
1	3.0 (0.4 - 11.5)	260
2	3.4 (0.7 - 12.5)	101
3	5.0 (1.1 - 12.7)	47
4	9.4 (2.9 - 14.0)	33
5	8.2 (3.5 - 12.4)	17
6	7.7 (5.4 - 13.6)	20
7	9.6 (6.8 - 13.2)	11
8	11.0 (8.2 - 12.1)	4
9	8.2 (6.7 - 9.1)	3
11	11.0 (11.0 - 11.0)	1

* Median (5% - 95%)

Figure 12 Number of follow-up visits and duration of follow-up



3.18 Genotype characteristics in the longitudinal cohort

Genetic information was available for 492 patients of the longitudinal cohort, in 5 patients of the longitudinal cohort the genetic test was not performed. Similarly to the baseline cohort most of patients carried at least one pathogenic variant, one third carried a confirmatory *MEFV* genotype according to Infever classification and 18.5% of patients had a negative or not informative genotype (Table 23, Figure 13). The presence of M694V variant was analyzed: M694V homozygous genotype was present in 9.7% of patients, 38.2% of patients carried one M694V variant alone or in compound heterozygosity with other variants and half of the patients did not carry any M694V variant.

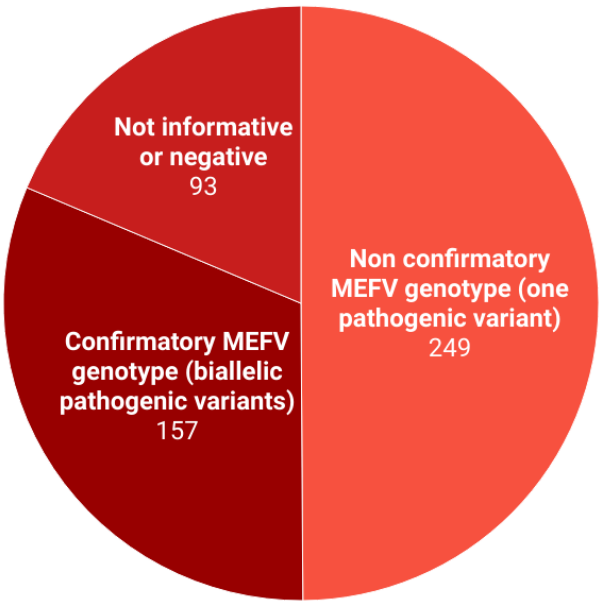
Table 23. Genotype classification according to Infevers in the longitudinal cohort

	N	**%**
Confirmatory *MEFV* genotype (biallelic pathogenic variant)	157	31.6 %
Non confirmatory *MEFV* genotype (monoallelic pathogenic variant)	242	48.7 %
Not informative or negative	93	18.5 %

Table 23. Genotype classification according to Infevers in the longitudinal cohort

	N	%
Presence of M694V variant in the cohort		
M694V homozygous	48	9.7
M694V heterozygous or compound heterozygous	190	38.2
M694V negative	254	51.1

Figure 13 Genotype according to Infever classification in the longitudinal cohort



3.19 Clinical characteristics of the longitudinal cohort

In the longitudinal cohort at disease onset a median of 12 episodes per year was reported (5-95th centile 3-25), with a median duration of episodes of 3 days (0.6-6), a regular frequency pattern of episodes was present in half of the patients.

The more frequent symptoms at disease onset were abdominal pain, fatigue, malaise, arthralgia, myalgia, chest pain and vomiting. In table 15 are listed the signs and symptoms at onset and at last follow-up.

During follow-up the symptoms associated with episodes changed significantly with marked reduction of all symptoms reported (Table 24 and Figure 14). Conversely, the rate of patients with regular frequency pattern of episodes did not significantly change, nor the presence of hepatomegaly, tenosynovitis, vertigo, conjunctivitis. The number of patients with amyloidosis did not increase during the follow-up.

Table 24. Signs and symptoms and characteristics of episodes

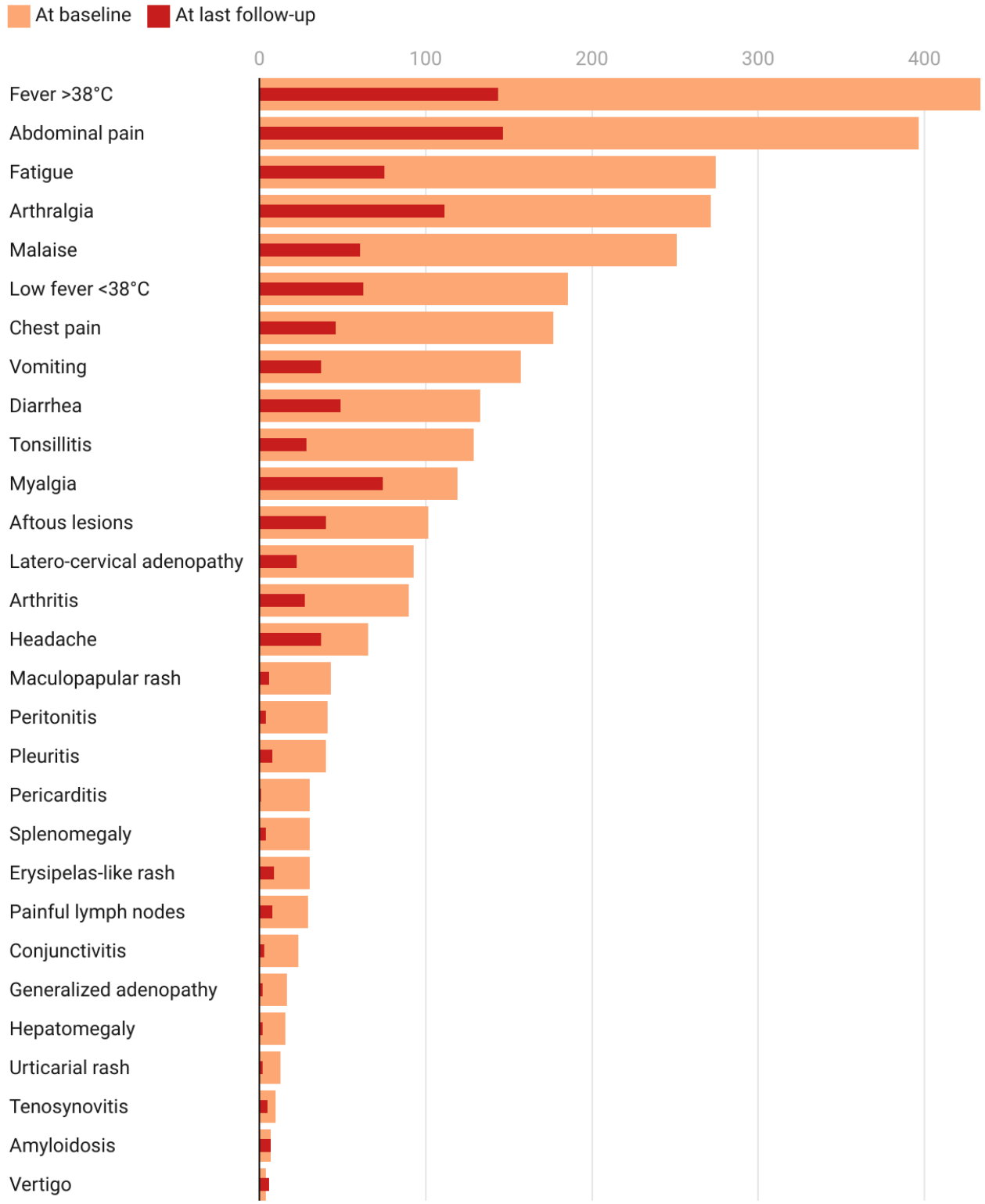
	At disease onset	**At last follow-up**	**p**
N of episodes per year	12.0 (3.0 - 25.0)	4.0 (1.0 - 24.0)	**<0.001**
Duration of episodes (days)	3.0 (0.6 - 6.0)	2.0 (0.5 - 5.0)	**<0.001**
Regular frequency pattern of episodes	233 (50%)	40 (17%)	0.005
Fever >38°C	434 (87.3%)	144 (28.9%)	**<0.001**
Often	156	85	
Always	278	59	
Low fever <38°C	186 (37.4%)	63 (12.7%)	**<0.001**
Often	137	48	
Always	49	15	
Fatigue	275 (55.3%)	76 (15.3%)	**<0.001**
Often	166	53	
Always	109	23	
Malaise	251 (50.5%)	61 (12.3%)	**<0.001**
Often	149	44	
Always	102	17	
Chest pain	177 (35.6%)	46 (9.2%)	**<0.001**
Often	119	37	
Always	58	9	
Pericarditis	31 (6.2%)	1 (0.2%)	**<0.001**
Often	23	1	
Always	8	-	
Pleuritis	40 (12.1%)	8 (1.6%)	**<0.001**
Often	53	7	
Always	7	1	
Peritonitis	41 (8.2%)	4 (0.8%)	**<0.001**
Often	39	3	
Always	2	1	

Table 24. Signs and symptoms and characteristics of episodes			
	At disease onset	**At last follow-up**	**p**
Abdominal pain	397 (79.9%)	147 (29.6%)	**<0.001**
Often	150	91	
Always	247	56	
Diarrhea	133 (26.8%)	49 (9.9%)	**<0.001**
Often	108	44	
Always	25	5	
Vomiting	158 (31.8%)	37 (7.4%)	**<0.001**
Often	125	34	
Always	33	3	
Generalized adenopathy	17 (3.4%)	2 (0.4%)	**<0.001**
Often	13	1	
Always	4	1	
Latero-cervical adenopathy	93 (18.7%)	23 (4,6%)	**<0.001**
Often	71	20	
Always	22	3	
Painful lymph nodes	30 (6.0%)	8 (1.6%)	**<0.001**
Often	28	6	
Always	2	2	
Tonsillitis	129 (25.9%)	29 (5.8%)	**<0.001**
Often	21	27	
Always	108	2	
Hepatomegaly	16 (3.2%)	2 (0.4%)	0.99
Often	12	1	
Always	4	1	
Splenomegaly	31 (6.3%)	4 (0.8%)	**<0.001**
Often	18	1	
Always	13	3	
Aftous lesions	102 (20.5%)	40 (8.0%)	**<0.001**
Often	78	33	
Always	24	7	
Maculopapular rash	43 (8.5%)	6 (1.2%)	**<0.001**
Often	39	6	
Always	3	-	
Urticarial rash	13 (2.6%)	2 (0.2%)	**<0.001**
Often	11	2	
Always	2	-	
Erysipelas-like rash	31 (6.2%)	9 (1.8%)	**<0.001**
Often	26	7	
Always	5	2	
Arthralgia	272 (54.7%)	112 (22.5%)	**<0.001**
Often	191	79	
Always	88	33	
Myalgia	191 (38.4%)	75 (15.1%)	**<0.001**
Often	146	64	
Always	45	11	
Arthritis	90 (18.1%)	28 (5.6%)	**<0.001**
Often	21	18	
Always	69	10	
Tenosynovitis	10 (2,0%)	5 (1.0%)	0.008

Table 24. Signs and symptoms and characteristics of episodes			
	At disease onset	**At last follow-up**	**p**
Often	8	4	
Always	2	1	
Headache	66 (13.3%)	37 (7.4%)	**<0.001**
Often	57	35	
Always	9	2	
Vertigo	4 (0.8%)	6 (1.2%)	0.975
Often	3	6	
Always	1	-	
Conjunctivitis	24 (4.8%)	3 (0.6%)	0.063
Often	23	3	
Always	1	-	
Amyloidosis	7 (1.4%)	7 (1.4%)	1.000

[1]Median (5% - 95%); n (%)

Figure 14 Signs and symptos variations during follow-up



Triggers were present in 17% of patients at baseline, while at last follow-up they were reported in 7.4% of patients (Table 25)

Table 25. Triggers at disease onset and at last follow-up

	At disease onset	At last follow-up
Total triggers	85 (17.1%)	37 (7.4%)
Cold	16 (3.2%)	3 (1.2%)
Stress	24 (4.8%)	21 (4.2%)
Vaccination	4 (0.8%)	1 (0.2%)
Infection	16 (3.2%)	11 (2.2%)
Exercise	28 (5.6%)	9 (1.8%)
Trauma	5 (1.0%)	2 (0.4%)
Food	4 (0.8%)	1 (0.2%)
Menstruations	13 (2.6%)	5 (1.0%)
Fatigue	27 (5.4%)	9 (1.8%)
Travel	12 (2.4%)	4 (0.8%)

[1]n (%)

3.20 Treatment

At enrollment in the registry 222 (44.7%) of patients received colchicine, 4 patients received anakinra and 1 patient received canakinumab, while 44.9% of patients were off therapy (Table 10). As expected, these percentage changed during the follow-up: at last follow-up most patients received colchicine with a median duration of treatment of 4.4 years (5-95th centile 0.4-15.4%). 24 patients received anakinra during the follow-up, but at last visit anakinra treatment was ongoing in only 3 patients. Canakinumab was prescribed to 44 patients during the follow-up and at last visit the 93.2% of patients were still receiving this drug, with a median duration of treatment of 1.8 years (5-95th centile 0.1 – 7.6). The number of patients off-therapy was elevated at baseline and markedly reduced during follow-up.

In table 26 are detailed the dosage received for colchicine, anakinra and canakinumab, both in absolute numbers and in mg pro kg. Frequency of canakinumab administration varied largely in the cohort, therefore we calculated the dosage received every four weeks to be able to analyze it.

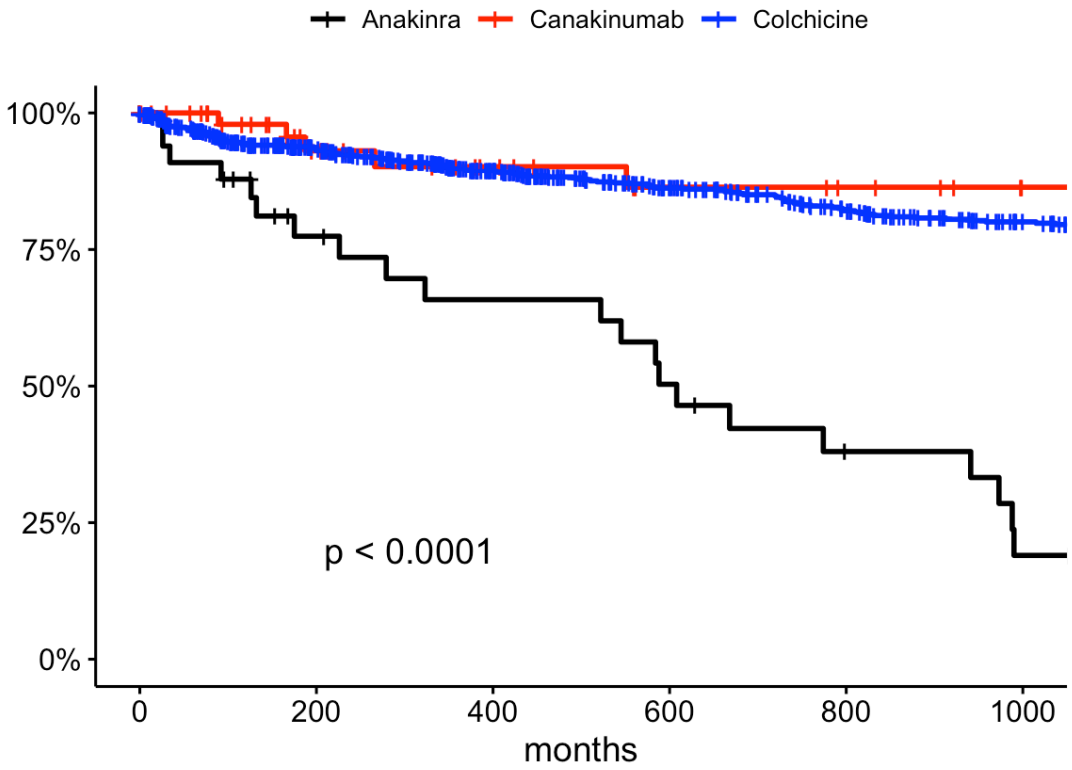
Mean colchicine dosage was 1 mg/day at baseline (25-75th centile 0.5 – 1.0) while at last follow-up was 1.1 (1 – 1.25), mean anakinra dosage was 51.8 mg/day (25-75th centile 27.7 – 81.3) while at last follow-up was 1.8 mg/day (1.4 – 2.1). Canakinumab dosage was 66.6 mg/4 weeks for the only patients in treatment at baseline, while at last follow-up the mean dosage was 125.4 mg/4 weeks (66.7 – 150.0). Figure 15 shows the survival on treatment over time for the different drugs with a clear difference between anakinra and the other two.

Table 26. Treatment in longitudinal cohort

	At baseline	At last follow-up
Off therapy/other treatment	223 (44.9%)	20 (4.0%)
Colchicine, n (%)	222 (44.7%)	475 (95.6%)
Ongoing, n (%)	186 (83.8%)	428 (90.1%)
Duration in years, median (range)	3.4 (0.2 – 17.1)	4.4 (0.4 – 15.4)
Dosage in mg/day, mean (25-75th centile)	0.9 (0.5 – 1.0)	1.1 (1 – 1.25)
Dosage in mg pro kg/day, mean (25-75th centile)	.024 (.015 - .029)	.025 (.016 - .031)
Anakinra, n (%)	4 (0.8%)	24 (4.8%)
Ongoing, n (%)	1 (25%)	3 (12.5%)
Duration in years, median (range)	3.9 (0.5 – 6.9)	1.8 (0.2 – 7.1)
Dosage in mg/day, mean (25-75th centile)	51.8 (27.7 – 81.3)	95.4 (100-100)
Dosage in mg pro kg/day, mean (25-75th centile)	1.6 (1.3 – 1.9)	1.8 (1.4 – 2.1)
Canakinumab, n (%)	1 (0.2%)	44 (8.9%)
Ongoing, n (%)	1 (100.0%)	41 (93.2%)
Duration in years, median (range)	8.9 (-)	1.8 (0.1 – 7.6)
Dosage in mg/4 weeks, mean (25-75th centile)	66.6 (-)	125.4 (66.7 – 150.0)

Dosage in mg pro kg/4 weeks, mean (25-75th c)	1.45 (-)	2.8 (1.5 – 3.2)

Figure 15 Survival analysis on treatment in the longitudinal cohort



We further evaluate the reasons of treatment withdrawal for the three drugs both in the period before the enrollment in Eurofever and in the longitudinal study period, as detailed in Table 27. Most frequent reasons of colchicine withdrawal were remission, inefficacy and intolerance. Anakinra was stopped mostly because of switch to canakinumab, inefficacy and intolerance, while canakinumab had a low rate of withdrawal with two stops of treatment for inefficacy and one for clinical remission.

Table 27. Reason of treatment withdrawal

	Colchicine		**Anakinra**		**Canakinumab**	
	Baseline	**Last FU**	**Baseline**	**Last FU**	**Baseline**	**Last FU**
Adverse events	-	1	-	-	-	-
Mild adverse event	3	3	-	2	-	-
Inefficacy	5	9	1	5	-	2
Intolerance	4	4	2	4	-	-
Patient choice	1	7		1	-	-
Pregnancy	1	-	-	-	-	-
Surgery	1	-	-	-	-	-
Chemotherapy for cancer	-	-	-	1	-	-

Switch to canakinumab	-	-	-	5	-	-
Remission	11	21	-	2	-	1
Improvement	1	-	-	-	-	-
Reason not known	-	2	-	1	-	-
Total	27	44	3	41	-	3

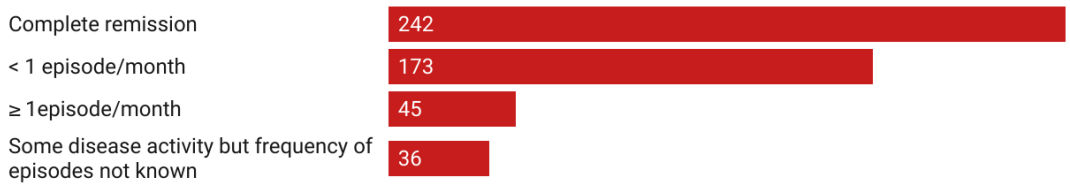
3.21 Disease activity

Disease activity was obviously available only for the longitudinal cohort. At last follow-up around half of patients were in complete remission, 51.3% had some disease activity: out of them 173 patients experienced <1 episode per month, 36 patients had some disease activity, but the frequency of episodes was unknown, and 46 patients experienced one or more episode per month (Table 28, Figure 16).

Table 28. Disease activity at last follow-up*	
Complete remission	242 (48.7%)
Incomplete response	255 (51.3%)
< 1 episode/month	173 (34.8%)
≥ 1episode/month	46 (9.2%)
Some disease activity but frequency of episodes not known	36 (7.2%)

**N (%)*

Figure 16 Disease activity at last follow-up



We analyzed disease activity at last follow-up according to genotype Infevers classification, as shown in Table 20 and Figure 17. More than half of patients with confirmatory and non-confirmatory genotype were in complete remission at last follow-up, a slightly lower rate was in remission among patients with negative genotype. No significant difference in disease activity was noticed between the groups of patients according to their genotype classification.

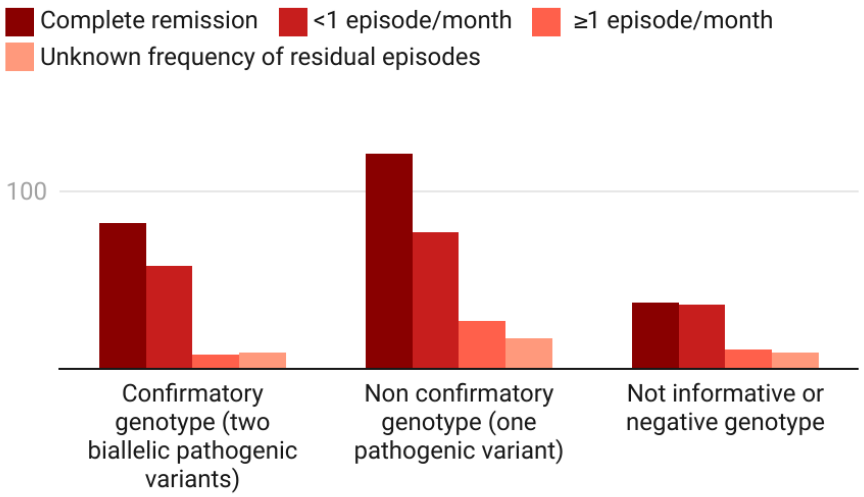
Table 29. Disease activity according to *MEFV* variant classification

	***MEFV* variant classification**			
	Confirmatory	**Non confirmatory**	**Negative**	**p**
Complete remission	82 (52%)	121 (50%)	37 (40%)	0.173
Incomplete response				

Table 29. Disease activity according to *MEFV* variant classification

	***MEFV* variant classification**			
	Confirmatory	**Non confirmatory**	**Negative**	**p**
<1 episode/month	58 (37%)	77 (32%)	36 (39%)	
Unknown frequency of residual episodes	8 (5.1%)	27 (11%)	11 (12%)	
≥1 episode/month	9 (5.7%)	17 (7.0%)	9 (9.7%)	

Figure 17 Disease activity and genotype classification



Similarly, we analyzed the disease activity at last follow-up according to the presence of *M694V* variant. No significant difference of treatment was noticed between groups (Table 30).

Table 30. Disease activity according to M694V variant presence

	M694V homozygous		**M694V heterozygous**		**M694V negative**		**p**
Complete remission	22	45.8%	90	47.4%	128	50.4%	
Incomplete response							0.408
<1 episode/month	22	45.8%	68	35.8%	81	31.9%	
≥1 episode/month	1	2.1%	20	10.5%	25	9.8%	

Table 30. Disease activity according to M694V variant presence

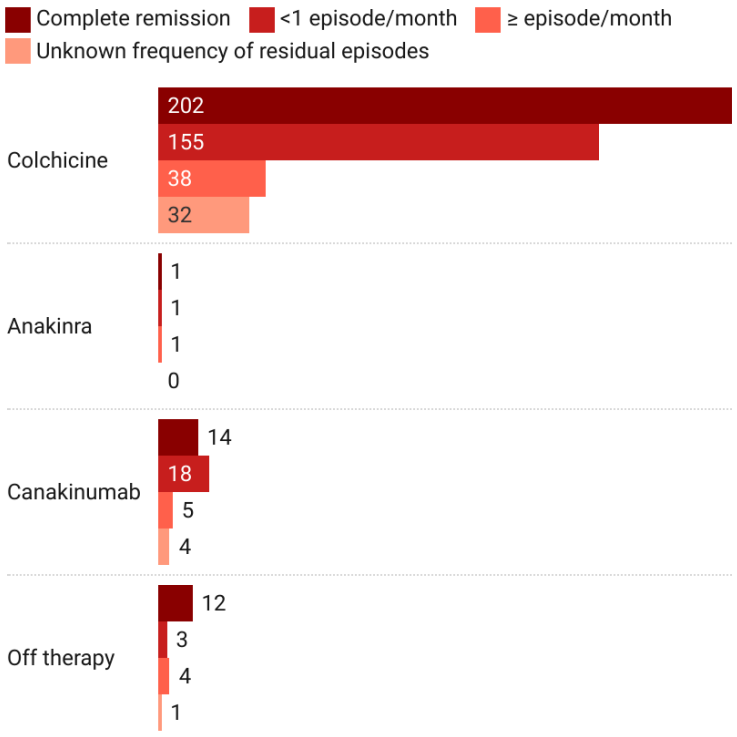
	M694V homozygous		M694V heterozygous		M694V negative		p
Unknown frequency of residual episodes	3	6.3%	12	6.3%	20	24.8%	

Table 31 and Table 18 shows the disease activity at last follow-up in the different groups receiving colchicine, anakinra, canakinumab and the group of patients without any treatment ongoing. Around half of patients in colchicine achieved complete remission, one third of patients in anakinra and canakinumab achieved complete response. Peculiarly rate of complete response was higher in patients off therapy.

Table 31. Disease activity at last follow-up according to treatment received

	Colchicine (N = 428)	Anakinra (N = 3)	Canakinumab (N = 41)	Off therapy (N= 20)
Complete remission	202 (47.2%)	1 (33.3%)	14 (32.6%)	12 (60.0%)
Incomplete response				
<1 episode/month	155 (36.2%)	1 (33.3%)	18 (41.9%)	3 (15.0%)
≥ episode/month	38 (8.9%)	1 (33.3%)	5 (11.6%)	4 (20.0%)
Unknown frequency of residual episodes	32 (7.5%)	0	4 (9.3%)	1 (5.0%)

Figure 18 Disease activity according to treatment received



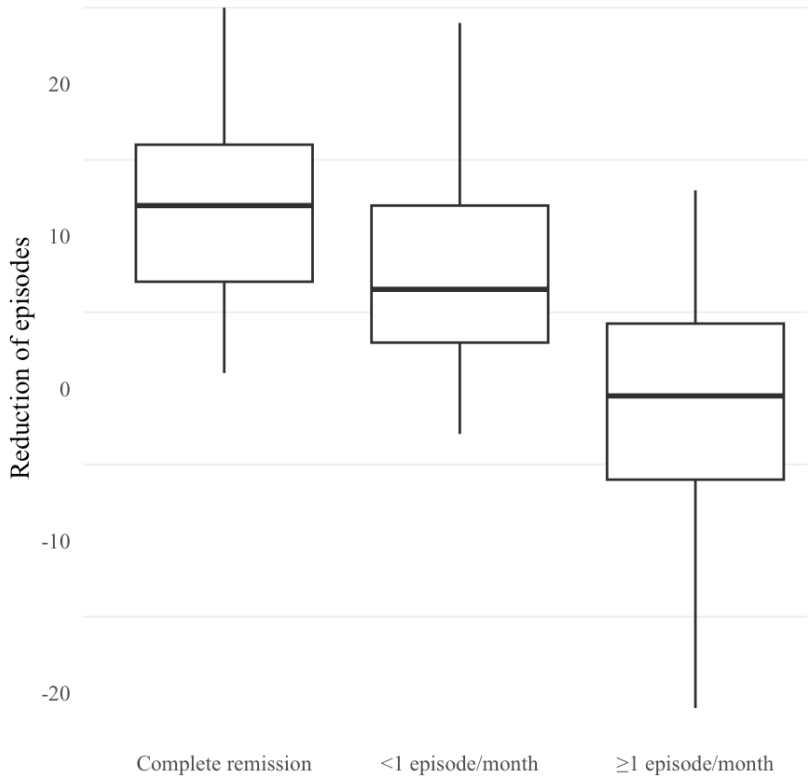
Moreover, we analyzed the change in frequency and duration of episodes in the cohort in relation to disease activity. As expected, the median reduction of episodes per year varied greatly between patients in complete remission and patients with incomplete response to treatment (Table 32, Figure 19). Conversely, the reduction of duration of episodes did not vary significatively between different groups of patients with incomplete response.

Table 31. Modification of frequency and duration of episodes during follow-up

	Complete remission, N = 242[1]	**< 1 episode/month, N = 173[1]**	**≥ 1 episode/month, N = 46[1]**	**p**
Reduction of episodes/year	12.0 (3.0 - 25.0)	6.5 (-1.0 - 23.0)	-0.5 (-18.1 - 13.0)	<0.001
Reduction of duration of episodes	-	0.0 (-2.0 - 3.0)	1.0 (-0.3 - 4.7)	0.487

[1]median (5-95th centile)

Figure 19 Reduction of episodes in relation to disease activity



We analyzed colchicine dosage of various treatment at last follow-up for colchicine, anakinra and canakinumab in relation to disease activity as detailed in Table 17. No significant difference was noticed in treatment dosage among groups with different disease activity.

In Table 32 we also reported the patients treated with colchicine that, at last follow-up, were still receiving the recommended starting dose of colchicine and receiving the max recommended dose defined by the EULAR recommendation (120). No significant difference was observed between disease activity groups.

Table 32. Treatment dosage at last follow-up in relation to disease activity

	Complete remission	**<1 episode/month**	**≥1 episode/month**	**Unknown frequency of residual episodes**	**p**
Colchicine mg/day[1]	1.0 (0.5 - 2.0)	1.0 (0.5 - 2.0)	1.0 (0.5 - 2.0)	1.0 (0.5 - 1.6)	.366
Colchicine mg pro kg/day[1]	.021 (.01-.044)	.026 (.012-.05)	.022 (.01-.051)	.023 (.013 – .045)	.047
Starting dose of colchicine[2]	126 (61%)	75 (48%)	21 (53%)	18 (53%)	.085
Max dose of colchicine[2]	1 (0.5%)	2 (1.3%)	1 (2.5%)	0 (0%)	.562
Anakinra mg/day[1]	75 (-)	100 (-)	100 (-)		.287
Anakinra mg pro kg/day[1]	1.42 (-)	1.3 (-)	1.1 (-)	0 (0%)	.655
Canakinumab mg/4 weeks[1]	131.3 (36.5 - 300.0)	150.0 (44.6 - 172.5)	85.0 (27.8 - 150.0)	150.0 (110.0 - 270.0)	.290
Canakinumab mg pro kg/4 weeks[1]	2.1 (0.8 - 8.4)	2.6 (0.8 - 6.7)	1.7 (0.5 - 2.8)	2.7 (1.7 - 4.8)	.085

[1]Median (5% - 95%); [2] N(%)

3.22 Compliance

Compliance is often a difficult aspect to tackle and assess by the physician, although extremely important in determining response to treatment. Compliance information was incomplete in the cohort because the specific form was added in Eurofever registry only in 2022.

125 patients of the longitudinal cohort had complete information about compliance, 115 were treated with colchicine, 71with anakinra and 9 with canakinumab (Table 33).

Optimal compliance (>90% of prescriptions) was achieved in 71.3% of patients with colchicine and 66.7% of patients with canakinumab. The one patient in anakinra declared optimal compliance to this drug.

Good compliance (50-80% of prescriptions) was achieved in 24.3% of patients treated with colchicine, and 33.3% of patients treated with canakinumab.

3.5% of patients treated with colchicine declared to have poor compliance (<50% of prescriptions) while only one patient declared to be not compliant at all to colchicine prescription.

Table 33. Compliance to treatment

	Colchicine	**Anakinra**	**Canakinumab**
Optimal compliance*	82 (71.3%)	1 (100%)	6 (66.7%)
Good compliance**	28 (24.3%)	0	3 (33.3%)
Poor compliance***	4 (3.5%)	0	0
Not compliant	1 (0.9%)	0	0
Total	115	1	9

*>90%, **50-90%, ***<50% of prescriptions

Moreover, we compared compliance to treatment and disease activity as detailed in Table 34.

Table 34. Compliance to treatment according to disease activity

Disease activity		**N**	**%**
Compliance to colchicine			
Optimal (>90%)	Complete remission	50	43.5 %

Table 34. Compliance to treatment according to disease activity			
	Disease activity	**N**	**%**
	<1 episode/month	24	20.9 %
	≥1 episode/month	2	1.7 %
	Unknown frequency of residual episodes	6	5.2 %
Good (50-90%)	Complete remission	12	10.4 %
	<1 episode/month	12	10.4 %
	≥1 episode/month	4	3.5 %
	Unknown frequency of residual episodes	0	0.0 %
Poor (<50%)	Complete remission	0	0.0 %
	<1 episode/month	4	3.5 %
	≥1 episode/month	0	0.0 %
	Unknown frequency of residual episodes	0	0.0 %
Not compliant	Complete remission	0	0.0 %
	<1 episode/month	1	0.9 %
	≥1 episode/month	0	0.0 %
	Unknown frequency of residual episodes	0	0.0 %
Compliance to anakinra			
Optimal (>90%)	Complete remission	0	0.0 %
	<1 episode/month	1	100.0 %
	≥1 episode/month	0	0.0 %
	Unknown frequency of residual episodes	0	0.0 %
Compliance to canakinumab			
Optimal (>90%)	Complete remission	3	33.3 %
	<1 episode/month	1	11.1 %
	≥1 episode/month	1	11.1 %
	Unknown frequency of residual episodes	1	11.1 %
Good (50-90%)	Complete remission	1	11.1 %
	<1 episode/month	1	11.1 %
	≥1 episode/month	1	11.1 %
	Unknown frequency of residual episodes	0	0.0 %

3.23 Quality-of-life

Similarly to compliance, quality-of-life information was available for a limited number of patients because the specific form was added to the registry only in 2022.

We had a complete quality-of-life information for eighty-eight patients, among them fifty-nine were in complete remission, twenty-eight had less than one episode per month, just one patient had more than one episode per month and two patients had some disease activity with unknown frequency of residual episodes.

In Table 35 and Figure 20-24 we detail the relationship between quality-of-life response and disease activity. All patients in complete remission showed optimal score in every item investigated, on the contrary patients with active disease reported some level of impact on quality-of-life aspects.

Table 35. Quality of life and disease activity

				Centile	
	Disease activity	**N**	**Median**	**5th**	**95th**
Level of activity of the illness by the physician in the last month (VAS scale)	Complete remission	59	0.00	0.000	0.00
	<1 episode/month	28	1.00	0.000	2.00
	≥1 episode/month	1	2.00	2.000	2.00
	Unknown frequency of residual episodes	2	3.25	0.775	5.72
Level of activity of the illness in the last month according to patient or family (VAS scale)	Complete remission	59	0.00	0.000	0.00
	<1 episode/month	28	1.00	0.000	3.65
	≥1 episode/month	1	3.00	3.000	3.00
	Unknown frequency of residual episodes	2	3.25	0.775	5.72
Level of tiredness in everyday life in the last month according to patient or family (VAS scale)	Complete remission	59	0.00	0.000	0.00
	<1 episode/month	28	1.00	0.000	2.00

Table 35. Quality of life and disease activity

				Centile	
	Disease activity	**N**	**Median**	**5th**	**95th**
	≥1 episode/month	1	0.00	0.000	0.00
	Unknown frequency of residual episodes	2	3.25	0.775	5.72
General subjective feeling considering all the ways the illness affects the life of the patient/family (VAS scale)	Complete remission	59	0.00	0.000	0.00
	<1 episode/month	28	1.00	0.000	4.00
	≥1 episode/month	1	2.00	2.000	2.00
	Unknown frequency of residual episodes	2	3.25	0.775	5.72
Number of day/work days lost in the month before the interview	Complete remission	50	0.00	0.000	0.00
	<1 episode/month	23	1	0.000	2.00
	≥1 episode/month	0	-	-	-
	Unknown frequency of residual episodes	2	0.00	0.000	0.00

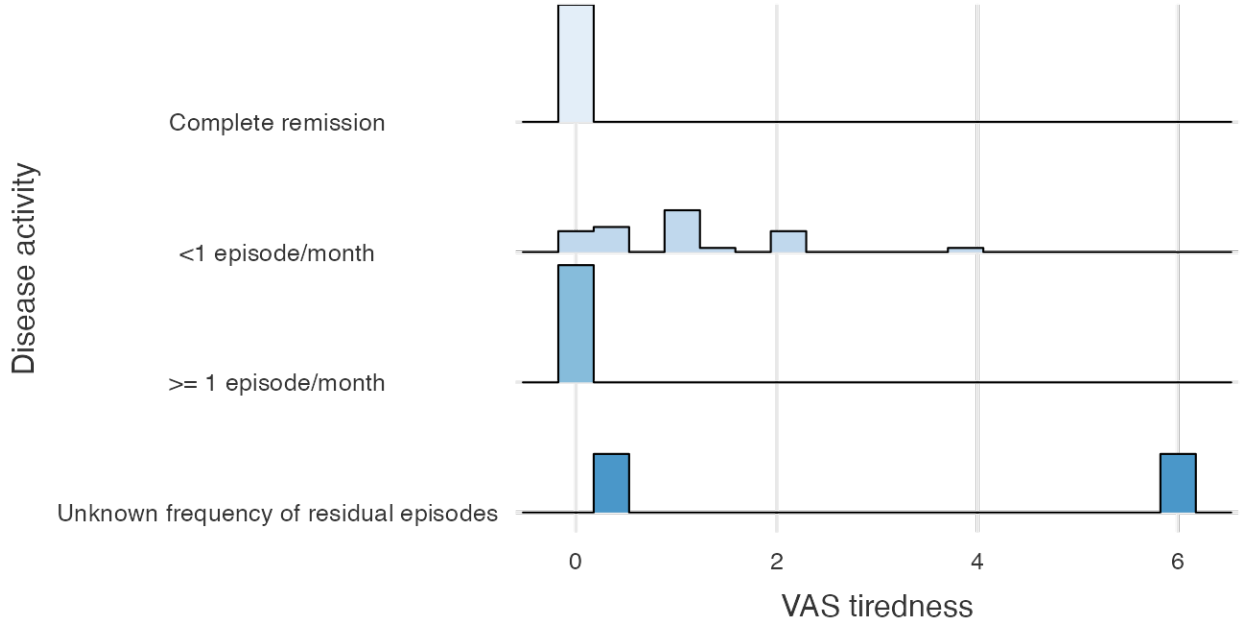


Figure 20 Level of tiredness in everyday life in the last month according to patient or family

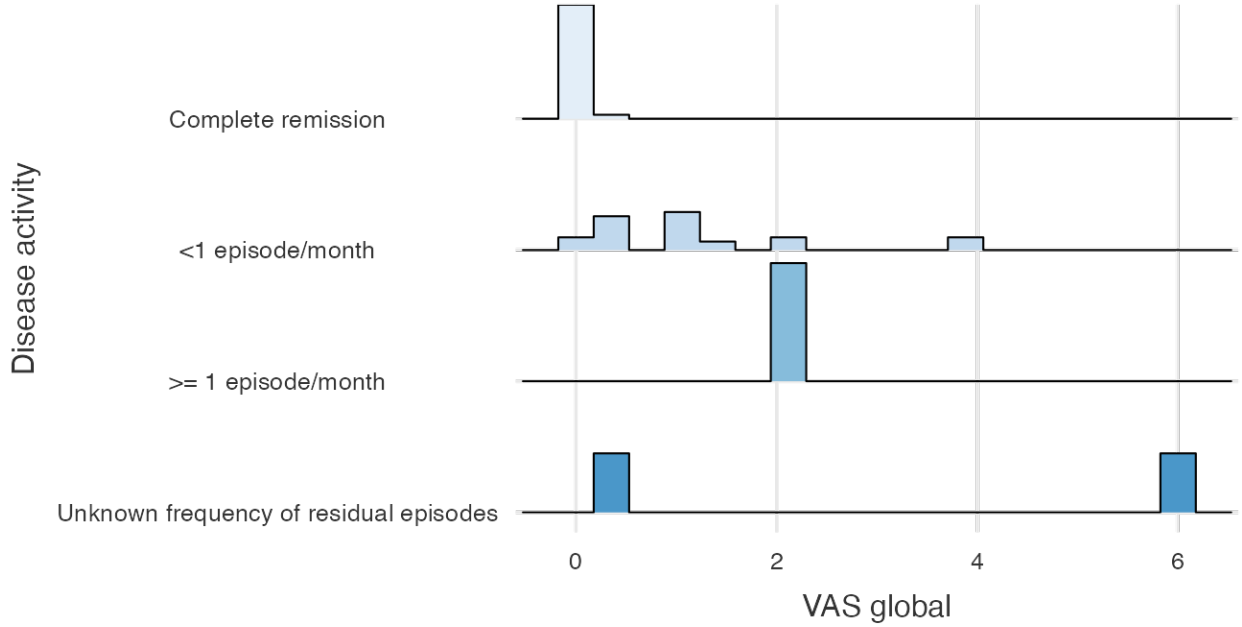


Figure 21 General subjective feeling considering all the ways the illness affects the life of the patient/family (VAS scale)

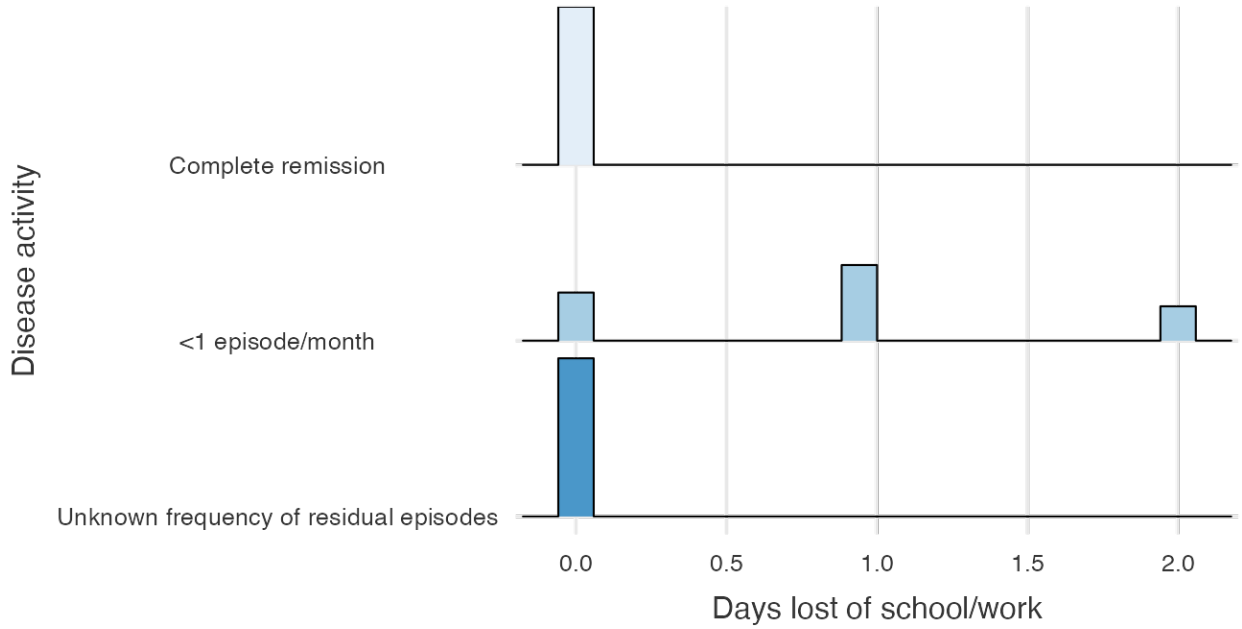


Figure 22 Number of day/work days lost in the month before the interview

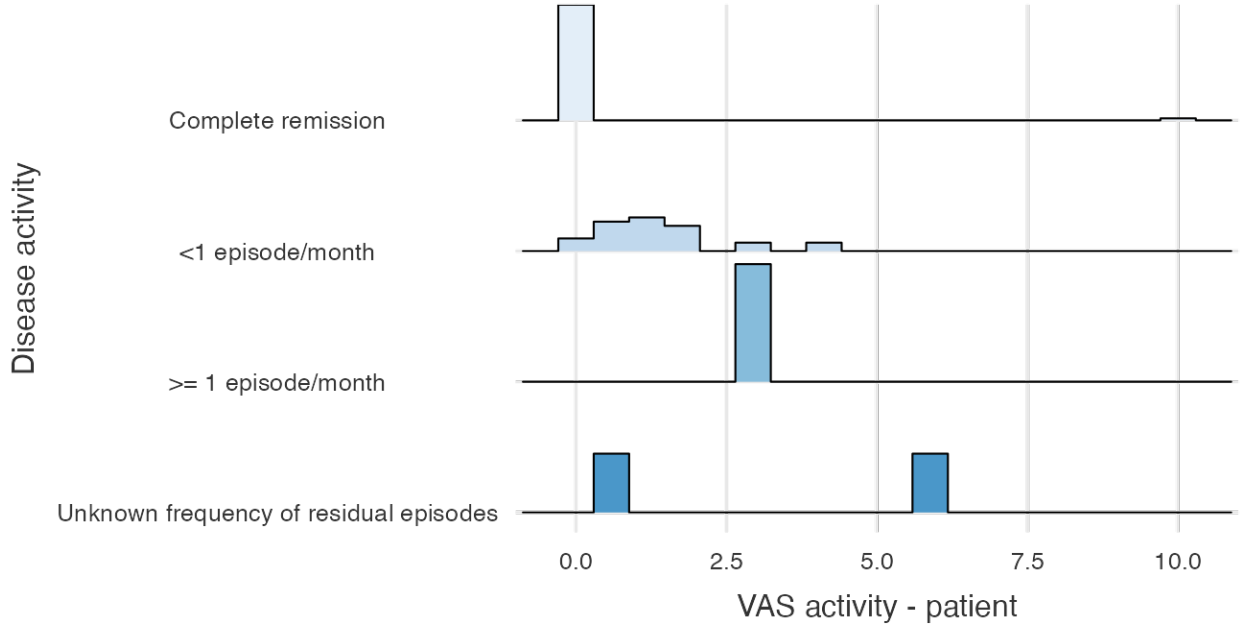


Figure 23 Level of activity of the illness in the last month according to patient or family (VAS scale)

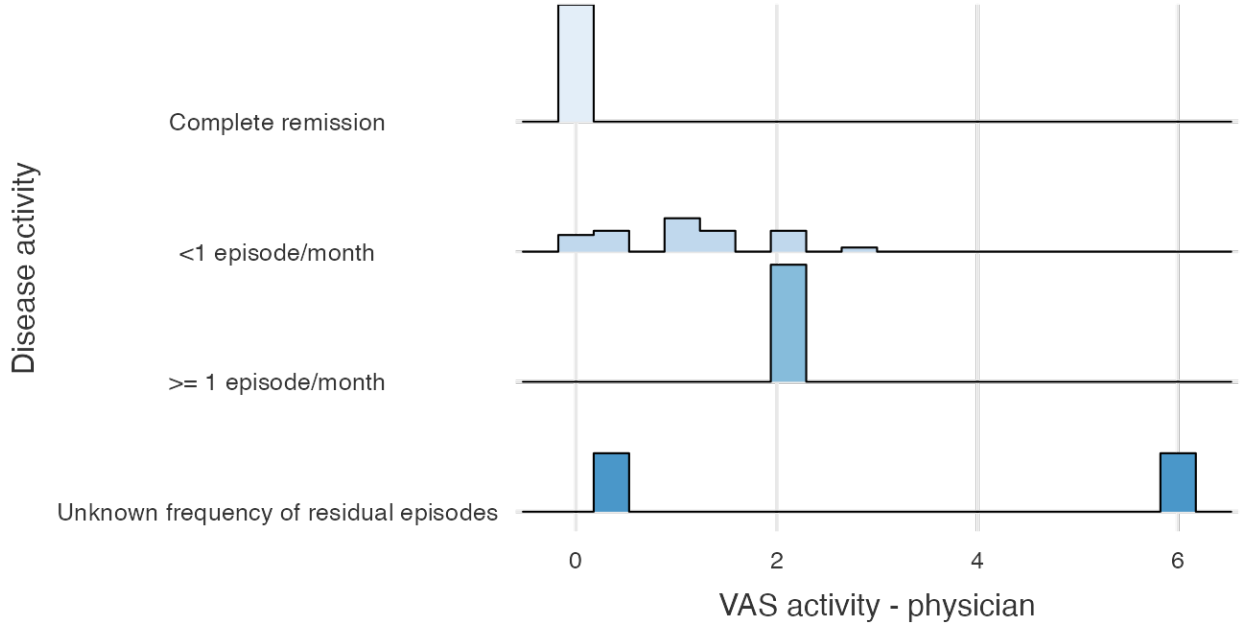


Figure 24 Level of activity of the illness by the physician in the last month (VAS scale)

3.24 Safety and adverse events

Safety data were analyzed in the longitudinal cohort. A total of 149 adverse events were reported during the follow-up of the longitudinal cohort. Most adverse events were reported in patients treated with colchicine, as expected for the frequency of this treatment in our cohort. Most frequent adverse events reported were infectious -mostly upper respiratory tract infection- and gastrointestinal, mostly abdominal pain alone or accompanied by vomiting or diarrhea. Summary of adverse events reported and the treatment received are shown in Table 36.

Table 36. Adverse event reported and treatment received at the time of occurrence

	Colchicine	**Anakinra**	**Canakinumab**	**Total**
Infectious AE	**44**	**1**	**4**	**46**
Abscess of skin	1	0	0	1
Upper respiratory tract infection	23	0	0	23
Pneumonia	6	0	1	7
Bronchitis	2	0	0	2
Cold sore reactivation	0	0	1	1
COVID-19 infection	2	0	1	2
Dacryoadenitis	1	0	0	1
Febrile neutropenia	1	0	1	1
Lymphadenitis	1	0	0	1
Impetigo	1	0	0	1
Parvovirus B19 infection	1	0	0	1
TBC (latent)	1	1	0	1
UTI	2	0	0	2
Varicella	2	0	0	2
Gastrointestinal and hepatic AE	**37**	**1**	**1**	**35**
Abdominal pain	6	0	0	6
Abdominal pain with vomiting and diarrhea	9	0	0	9
Regional enteritis of large intestine	1	0	0	1
Abdominal pain with peritonism	1	0	0	1
Diarrhea	7	0	1	7
Erosive gastritis	2	0	0	2
Hypertransaminasemia	6	1	0	6
Vomiting	1	0	0	1
Respiratory AE	**3**	**0**	**0**	**3**
Asthma	2	0	0	2
COBP	1	0	0	1
Lymphatic tissues AE	**2**	**0**	**0**	**2**
Splenic granuloma	1	0	0	1
Splenomegaly	1	0	0	1
Skin and allergic AE	**10**	**0**	**2**	**9**
Acne	1	0	0	1
Skin rash	2	0	1	3

Table 36. Adverse event reported and treatment received at the time of occurrence

	Colchicine	Anakinra	Canakinumab	Total
Recurrent oral aphtosis	4	0	0	4
Urticarial rash	1	0	0	1
Allergic conjunctivitis	1	0	1	1
Allergic rhinitis	1	0	0	1
Osteo-muscular AE	**14**	**0**	**0**	**14**
CPK increased	4	0	0	4
Arthralgia	4	0	0	4
Myalgia	1	0	0	1
Tendonitis	2	0	0	2
Vertebral collapse	1	0	0	1
Fracture	1	0	0	1
Arthrosis	1	0	0	1
Cardiovascular AE	**6**	**0**	**0**	**6**
Chest pain	1	0	0	1
Mitral valve prolapse	1	0	0	1
Myopericarditis	2	0	0	2
STEMI	1	0	0	1
Primary hypertension	1	0	0	1
Neurologic AE	14	0	0	14
Headache	9	0	0	9
Febrile seizure	1	0	0	1
Psychotic depression	1	0	0	1
Peripheral sensory neuropathy	2	0	0	2
Vertigo	1	0	0	1
Renal and genitourinary AE	6	0	0	6
Renal colic	1	0	0	1
Nocturnal enuresis	1	0	0	1
Ovary cyst	1	0	0	1
Microalbuminuria	1	0	0	1
Neoplastic AE	2	0	0	2
Breast cancer	1	0	0	1
Whartin’s tumor	1	0	0	1
ENT and ocular AE	5	0	0	5
Vocal cord nodule	1	0	0	1
Epistaxis	1	0	0	1
Intermediate uveitis	1	0	0	1
Recurrent chalazion	1	0	0	1
Optic neuropathy	1	0	0	1
Other	7	0	1	7
Macrophage activation syndrome	1	0	0	1
Enoch-Schoenlein purpura	3	0	0	3
Fatigue	2	0	1	2
Weight loss	1	0	0	1

25 adverse events were reported as serious. The patients were all receiving colchicine when these adverse events were reported, 4 patients received also canakinumab and one patient received anakinra in concomitance with colchicine.

In 15 patients the adverse events were related to colchicine, mostly with gastrointestinal symptoms. Only one patient was treated with canakinumab at the same time (canakinumab). Cause-effect relationship with colchicine was judged definite in 3 cases, probable in 6 cases. Only one adverse event was considerate severe while the others were reported as moderate. In 5 cases the drug was discontinued and in 2 cases the dose was reduced, in the other cases no action was taken by the physician (Table 37).

As detailed in table 38, 18 of the serious adverse events required hospitalization (72%), 4 required surgery (16%). At the last follow-up visit 22 of them (88%) were resolved, while 3 (12%) were improved and in 3 cases the problem was persisting. Resulting disability was reported in 6 cases (24%).

Table 37. Adverse event related to colchicine

Adverse event	Intensity	Relation to colchicine	Hospitalisation	Action taken	Other treatment
Abdominal pain	Moderate	Definite		Drug discontinued	
Abdominal pain with vomiting and diarrhea	Moderate	Possible		None	
Abdominal pain with vomiting and diarrhea	Moderate	Definite		Drug discontinued	
Diarrhea	Moderate	Probable		None	
Diarrhea	Moderate	Probable		Drug discontinued	
Diarrhea	Severe	Possible		Dose reduced	
Fatigue	Moderate	Possible		Drug discontinued	
Diarrhea	Moderate	Probable		Drug discontinued	
Hypertransaminasemia	Moderate	Probable		None	
Upper respiratory infection	Moderate	Possible		None	
Pneumonia	Moderate	Possible	Yes	None	
Pneumonia	Moderate	Possible		None	
Rash	Moderate	Probable		None	
Vertigo	Moderate	Possible		None	
Vomiting	Moderate	Definite		Dose reduced	Canakinumab

Table 38. Serious adverse events reported

Serious AE	Intensity	Hospitalisation	Required surgery	Outcome	Disability	Treatment received
Abdominal pain with peritonism	Severe	Yes		Resolved		Colchicine
Acute respiratory failure	Very severe	Yes		Resolved		Colchicine
Breast cancer	Moderate			Resolved		Colchicine, anakinra
COBP	Moderate			Persisting		Colchicine
COVID-19	Moderate			Resolved	Yes	Colchicine, canakinumab
Cyst ovary	Moderate	Yes		Resolved		Colchicine
Erosive gastritis	Moderate			Persisting	Yes	Colchicine
Febrile neutropenia	Mild	Yes		Resolved		Colchicine, canakinumab
Febrile seizure	Moderate	Yes		Resolved		Colchicine
Henoch Shonlein purpura	Moderate	Yes		Resolved		Colchicine
Henoch-Schonlein purpura	Severe	Yes		Resolved		Colchicine
Henoch-Schonlein purpura	Moderate	Yes		Resolved		Colchicine, canakinumab
Macrophage activation syndrome in sjia comorbidities	Severe	Yes		Resolved		Colchicine
Middle ear infection	Moderate	Yes		Resolved		Colchicine
Myopericarditis	Moderate	Yes		Improved		Colchicine
Optic neuropathy	Severe	Yes		Resolved		Colchicine
Osteoarthritis	Moderate		Yes	Persisting	Yes	Colchicine
Peripheral sensory neuropathy	Severe		Yes	Improved	Yes	Colchicine
Pneumonia	Moderate	Yes		Resolved		Colchicine, canakinumab
Pneumonia	Moderate	Yes		Resolved		Colchicine

Table 38. Serious adverse events reported

Pneumonia	Moderate	Yes		Resolved		Colchicine
Pneumonia	Moderate	Yes	Yes	Resolved		Colchicine
Regional enteritis of large intestine	Moderate	Yes		Improved	Yes	Colchicine
					Yes	
Miocardial infarction	Severe	Yes	Yes	Resolved		Colchicine
Warthin's tumour	Moderate			Resolved		Colchicine

V. Conclusions

We described the baseline clinical features and disease outcome of an international longitudinal cohort of FMF patients, and we observed their changes over time.

Half of the patients in the cohort displayed some degree of disease activity, 9.2% of them displayed more than 1 episode per month, thus we can consider them as resistant, according to the EULAR recommendation that defines resistance as one or more attacks per month in compliant patients who had been receiving the maximally tolerated dose for at least 6 months.

As expected, during the follow-up we noticed a marked modification of treatment received by the cohort: the number of off-therapy patients decreased, while the rate of patients treated both with colchicine and with anti-IL1 increased. Among the last group, we observed an increase in patients treated with both anakinra and canakinumab, but the rate of treatment withdrawal was very different between the two groups: while only 12.5% treated with anakinra was still on the drugs at last follow-up, most patients treated with canakinumab were still receiving the treatment at the last visit.

Dosage of various treatment was examined in detail. The vast majority of patients in the cohort resulted to be under-treated with colchicine, even in the group of patients with some degree of disease activity. The dose of colchicine was overall relatively low, with a median of 1 mg/day (1 – 1.25), there were no significant change between the colchicine dosage at baseline and follow-up. This is confirmed by the observation that the majority of patients were still receiving the recommended starting dose of colchicine and very few received the maximum dosage recommended (120). Interestingly, the median dosage of colchicine in the group with active disease did not differ from patients in complete disease; patients with disease activity had the same probability of receiving the starting dose of colchicine than patients in remission.

The registry gave also the possibility to calculate the per kilo dose of colchicine. In our cohort, the mean dose of colchicine was 0.024 (0.016 – 0.031), without significant variations between groups with different level of activity. This value is lower than the one reported by Özkaya et

al. who showed that a dose of colchicine up to 0.03 - 0.07 mg/kg/day may be required to achieve a complete disease control (122). Many factors may contribute to the general tendency for the underdosage of colchicine in the present cohort, including intolerance, lack of compliance and adverse events. The same tendency for the underdosage of colchicine was observed for canakinumab: mean dosage was 125.4 (66.7 – 150) and pro kg dosage was 2.8 (1.5 – 3.2), with little variation between groups with different disease activity.

Compliance was overall satisfactory both for colchicine and canakinumab. Adverse events reported during treatment were mostly non serious and imposed the discontinuation of colchicine in a few cases. No adverse event was directly attributed by the physician to canakinumab or anakinra. Obviously, some failed attempts to increase the dosage due to intolerance or mild adverse events may have not been recorded in the registry. This could be a possible cause for the tendency not to increase the dose despite a partial control of the disease in some patients.

Moreover, most of the patients in the registry came from Eastern Mediterranean countries, where the severity of the disease is generally milder (7,148–150). The milder course of disease could explain the lower doses observed in the present study in respect to other studies deriving from Middle-East countries (151). In addition, we observed that a significant change in episodes between baseline and last follow-up: both in frequency, duration and in the clinical characteristics of them: rates of signs and symptoms decreased significantly during follow-up. It is therefore plausible that the global satisfaction for the overall control of the disease overcame the fear of possible drug-related side effects in case of dose increase.

The analysis of quality-of-life assessment confirm this impression: patients with disease activity shows some sort of impact on quality of life but median score was overall low. Indeed, a limit of this study is the incomplete data regarding quality-of-life. Besides, no specific instruments to quantify the health-related quality of life have been developed for autoinflammatory diseases, so far. It is conceivable that the longitudinal collection of these relevant aspects with more detailed instruments, such as 36-item Short Form (SF-36) and Childhood Health Assessment Questionnaire (CHAQ) at each follow-up visit will provide further insights on this issue.

In conclusion, the longitudinal collection of data in our study allows a more detailed and punctual picture in respect to previous studies based mainly on retrospective data. Even if a small fraction of patients (9.2%) presents a number of disease flares consistent with the definition of colchicine resistance according to EULAR criteria, a relevant percentage of

patients displays partial response despite treatment with a limited impact on the daily activities. Our study shows a general tendency of under-dosing colchicine.

The Eurofever centers are currently attempting to expand and complete the data of the FMF cohort, obtaining more information about treatment, compliance and quality of life, both in European and non-European country.

This effort will allow us to analyze the actual differences in the disease activity and prescription attitudes among different countries, the characteristics of FMF patients needing an IL-1 treatment and the impact of different therapeutic regimens on the disease course.

VI. Bibliography

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