ORIGINAL ARTICLE

Differentiating children with familial Mediterranean fever from other recurrent fever syndromes: The utility of new Eurofever/PRINTO classification criteria

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ABSTRACT

Objectives: In this study, we aimed to investigate the performance of Eurofever Registry and the Paediatric Rheumatology International Trials Organisation (PRINTO) classification criteria in pediatric patients with familial Mediterranean fever (FMF).

Patients and methods: This retrospective, cross-sectional study included a total of 130 pediatric FMF patients (67 males, 63 females; mean age: 12.4±3.6 years; range, 2.5 to 17.7 years) with at least one *M694V* mutation in *MEFV* gene between July 2010 and July 2019. Demographic features and disease characteristics were recorded. The control group was consisted of 41 patients (19 males, 22 females; mean age: 7.8±4.0 years; range, 2.1 to 17.8 years) with other hereditary autoinflammatory diseases (AIDs), including periodic fevers with aphthous stomatitis, pharyngitis, and adenitis syndrome (n=30), mevalonate kinase deficiency (n=9), and tumor necrosis factor receptor-associated periodic syndrome (n=2). Sensitivity and specificity of the Eurofever/PRINTO classification criteria were calculated.

Results: The sensitivity and specificity were 97.7% and 56.1% for Yalcinkaya-Ozen criteria, respectively and 93.1% and 90.2% for Tel Hashomer criteria, respectively. The Eurofever/PRINTO classification criteria reached a sensitivity and specificity of 94.6% and 82.9% and 93.1% and 80.5%, respectively, when genetic plus clinical criteria and clinical-only criteria were applied.

Conclusion: The Eurofever/PRINTO classification criteria have a comparable sensitivity for avoidance of FMF underdiagnosis in childhood. The Yalcinkaya-Ozen criteria have the highest sensitivity without a significant specificity. The Tel Hashomer criteria and Eurofever/PRINTO classification criteria were superior to Yalcinkaya-Ozen criteria to differentiate FMF from other AIDs, thus leading to less complications relevant to underdiagnosis of other AIDs.

Keywords: Classification, criteria, familial Mediterranean fever, pediatric.

Familial Mediterranean fever (FMF) is known as the most common hereditary autoinflammatory disease (AID) worldwide caused by pathogenic mutations in the *MEditerranean FeVer (MEFV)* gene.[1,2] The causative *MEFV* gene, firstly described in 1997, is located on chromosome 16, and the majority of the patients have biallelic *MEFV* mutations. By altering the function of pyrin protein, which is commonly expressed in neutrophils, these mutations lead to elevated amounts of interleukin-1 (IL-1) and excessive inflammation.[3,4] Typical clinical manifestations are

self-limiting attacks of recurrent fever, abdominal pain, arthralgia, and chest pain.[1,2] Although genetic analysis of *MEFV* gene supports the diagnosis of FMF, it is still recommended to diagnose the patients clinically.[5] There are several proposed criteria for adults and one for pediatric population; however, they cannot reach both high sensitivity and specificity at the same time.[6-9] Moreover, these criteria lack specificity while differentiating FMF from other recurrent fever syndromes, including periodic fever with aphthous stomatitis, pharyngitis, and adenitis (PFAPA) syndrome, tumor necrosis factor receptor-associated periodic syndrome (TRAPS), cryopyrin-associated periodic syndrome (CAPS), and mevalonate kinase deficiency (MKD).[9] Therefore, a new set of criteria have been recently announced for each AID by the Eurofever Registry and the Paediatric Rheumatology International Trials Organisation (PRINTO).[10,11] They introduced two sets of criteria for FMF, of which one includes genetic and clinical variables, the other includes clinical variables only.[11]

Two recent studies evaluated the performance of Eurofever/PRINTO classification criteria in pediatric FMF patients.[12,13] Due to the discrepancy between the results of these studies, we consider that the criteria should be studied further, particularly for the purpose of clarifying the possible avoidance of misdiagnosis of other AIDs in clinical practice. We, therefore, aimed to evaluate and compare the sensitivity and specificity of Eurofever/PRINTO classification criteria with two other diagnostic criteria in our pediatric FMF cohort.

PATIENTS AND METHODS

This single-center, retrospective, cross-sectional study was conducted at Çukurova University Faculty of Medicine, Department of Pediatric Rheumatology between July 2010 and July 2019. A total of 130 pediatric FMF patients (67 males, 63 females; mean age: 12.4±3.6 years; range, 2.5 to 17.7 years) with at least one *M694V* mutation in *MEFV* gene, and clinically diagnosed as having FMF, by a single physician. Patients with less than six-month follow-up were excluded from the study. Demographic features including age at onset of symptoms, age at

diagnosis, sex, and clinical properties were retrospectively collected from medical files of the patients. Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and serum amyloid A (SAA), obtained during an inflammatory attack, were also recorded retrospectively. The *MEFV* gene analysis was performed by next-generation sequencing (NGS) platform (Illumina®, MiSeq System, Illumina, Inc., San Diego, CA, USA).

The control group consisted of 41 patients (19 males, 22 females; mean age: 7.8±4.0 years; range, 2.1 to 17.8 years) with other AIDs, including PFAPA (n=30), MKD (n=9), and TRAPS (n=2). All participants in the control group underwent *MEFV* gene analysis and did not reveal confirmative genotype in any of them. The diagnosis of PFAPA was supported by the modified Marshall criteria.[14] The diagnosis of MKD and TRAPS were confirmed by NGS of MVK and *TNFRSF1A* genes. The Tel Hashomer, Yalcinkaya-Ozen and Eurofever/PRINTO criteria were applied to both FMF patients and control group.

A written informed consent was obtained from each parent and/or legal guardians of the patient. The study protocol was approved by the Çukurova University Faculty of Medicine Ethics Committee (No: 94/7, Date: 6/12/2019). The study was conducted in accordance with the principles of the Declaration of Helsinki.

Statistical analysis

Statistical analysis was performed using the IBM SPSS version 20.0 software (IBM Corp., Armonk, NY, USA). Continuous variables were presented in mean ± standard deviation (SD) or median (min-max), while categorical variables were presented in number and frequency. The Kolmogorov-Smirnov test was used to confirm the normal distribution. Categorical data were compared between two groups using the chi-square test. Continuous data were compared using the Student's t-test. The sensitivity and specificity of each set of criteria were assessed using the Kappa (κ) method. A *p* value of <0.05 was considered statistically significant.

RESULTS

Demographic and clinical features of the participants are summarized in

Table 1. Demographic data and clinical criteria between FMF and control group

	n	%	Mean±SD	Median	Min-Max	n	%	Mean±SD	Median	Min-Max	p
Age at symptom onset (year)			3.9±2.9	3.06	0.3-14.3			1.8±1.7	1.42	0.1-17.1	**0.001**
Age at diagnosis (year)			6.3±3.4					6.9±3.8			0.414
Age at study enrollment (year)			12.4±3.6					7.8±4.0			**0.001**
Sex											
Female	63	48.5				22	53.7				0.595
Criteria items											
Fever	123	94.6				41	100				0.141
Fever duration 1-3 days	113	86.9				18	43.9				**0.001**
Abdominal pain	116	89.2				30	73.2				**0.020**
Arthritis	44	33.8				4	9.8				**0.002**
Chest pain	24	18.5				2	4.9				**0.024**
Aphthous stomatitis	5	3.8				16	39				**0.001**
Painful lymph nodes	13	10				28	68.3				**0.001**
Urticarial rash	8	6.2				5	12.2				0.173
Maculopapular rash	3	2.3				9	22				**0.001**
Acute phase reactants during an attack									32	3-84	0.117
ESR (mm/1st h)				37.5	2-120				28.2	10-248	**0.001**
CRP (mg/L)				72	13.2-225				12900	5100-28350	0.071
Leukocytes (/mm^3)				10800	4800-39700				153	30-1510	0.077
SAA (ng/mL)				712	11-1110						
Fulfilment of criteria											
Tel Hashomer	121	93.1				4	9.8				**0.001**
Yalcinkaya-Ozen	127	97.7				18	43.9				**0.001**
Eurofever/PRINTO-G plus C	123	94.6				7	17.1				**0.001**
Eurofever/PRINTO-C	121	93.1				8	19.5				**0.001**

FMF: Familial Mediterranean Fever; SD: Standard deviation; ESR: Erythrocyte sedimentation rate; CRP: C-reactive protein; SAA: Serum amyloid A; PRINTO: Paediatric Rheumatology International Trials Organisation; G: Genetic; C: Clinical.

Table 1. Of 130 FMF patients, 83 (63.8%) were homozygous and 47 were heterozygous for the *M694V* mutation in *MEFV* gene. Age at symptom onset and study enrollment was significantly higher in FMF group. Although frequency of fever did not significantly differ between the two groups, fever duration of one to three days was statistically more common in the FMF group. Among the clinical symptoms, abdominal pain, arthritis and chest pain were more frequent during an inflammatory attack in the FMF group. However, aphthous stomatitis, painful lymph nodes, and maculopapular rash were more frequent in the control group (p=0.001). Besides, acute phase responses did not significantly differ between the two groups, except for that CRP was statistically higher in the FMF group.

The fulfillment of each set of criteria was statistically higher in the FMF group. Only four control patients were classified as FMF according to the Tel Hashomer criteria and two of them had PFAPA, one had MKD, and another had TRAPS. Eighteen of control patients were classified as

Table 2. Sensitivity and specificity results of Eurofever/PRINTO classification criteria, Yalcinkaya-Ozen criteria and Tel Hashomer criteria

Criteria	Sensitivity (%)	Specificity (%)
Eurofever/PRINTO classification criteria		
Genetic plus clinical criteria	94.6 (123/130)	82.9 (34/41)
Clinical criteria	93.1 (121/130)	80.5 (33/41)
Yalcinkaya-Ozen criteria	97.7 (127/130)	56.1 (23/41)
Tel Hashomer criteria	93.1 (121/130)	90.2 (37/41)

PRINTO: Paediatric Rheumatology International Trials Organisation.

Table 3. Evelution of sensitivity of Eurofever/PRINTO classification criteria, Yalcinkaya-Ozen criteria and Tel Hashomer criteria according to genotype

Criteria	Sensitivity (%)	
	Homozygote *M694V* mutation	Heterozygote *M694V* mutation
Eurofever/PRINTO classification criteria		
Genetic plus clinical criteria	100 (83/83)	85.1 (40/47)
Clinical criteria	94 (78/83)	91.5 (43/47)
Yalcinkaya-Ozen criteria	100 (83/83)	93.6 (44/47)
Tel Hashomer criteria	96.4 (80/83)	87.2 (41/47)

PRINTO: Paediatric Rheumatology International Trials Organisation.

FMF according to the Yalcinkaya-Ozen criteria, 11 had PFAPA, six had MKD, and one had TRAPS. Besides, five PFAPA patients and two MKD patients were misclassified as FMF according to the Eurofever/PRINTO classification criteria (genetic and clinical). When the clinical Eurofever/PRINTO classification criteria were applied to the participants, seven PFAPA and one MKD patients were misclassified as having FMF. The sensitivity and specificity values are shown in Table 2. The Yalcinkaya-Ozen criteria reached the highest sensitivity (97.7%) and the lowest specificity (56.1%). The highest specificity was achieved by the Tel Hashomer criteria.

The sensitivity was also evaluated by dividing FMF patients according to the genotype as homozygous *M694V* and heterozygous *M694V*. While the Eurofever/PRINTO classification criteria (genetic and clinical) and Yalcinkaya-Ozen criteria shared the same 100% sensitivity in homozygous patients, the Yalcinkaya-Ozen and only clinical Eurofever/PRINTO classification criteria reached the top two with 93.6% and 91.5% sensitivity, respectively, in patients with heterozygous *M694V* (Table 3).

DISCUSSION

The present study showed that sensitivity of all three criteria varied between 93.1 and 97.7%; however, their specificities were in a wide range between 56.1 and 90.2%. Although the Yalcinkaya-Ozen criteria reached the highest sensitivity (97.9%), it did lack a satisfying specificity (56.1%). Since the specificity of the Yalcinkaya-Ozen criteria was the lowest, it may

have led to significant misdiagnosis of other AIDs. With acceptable sensitivity rates, other criteria had a higher specificity, of which Tel Hashomer criteria had the highest.

In the Eurofever/PRINTO study, genetic plus clinical criteria yielded the sensitivity and specificity of 94% and 95% for FMF, whereas clinical-only criteria resulted in the sensitivity and specificity of 91% and 92% for FMF, respectively.[11] Latter studies also found a favorable diagnostic accuracy for the new classification criteria.[12,13] Interestingly, the study by Sag et al.[12] revealed that the new criteria had the highest sensitivity, but the lowest specificity among three criteria. On the contrary, the Tel Hashomer criteria reached the highest specificity, but showed the lowest sensitivity. Another study including a large number of genetically heterogeneous pediatric FMF patients having insignificant mutations and a distinct control group examined genetically unclassified AIDs.[13] The authors found sensitivity rates between 91.1 and 99.3% for the Livneh criteria, Yalcinkaya-Ozen criteria, and Eurofever/PRINTO criteria; however, the Tel Hashomer criteria had the lowest (82.6%) sensitivity. In this study, the sensitivity of the Tel Hashomer criteria was lower than the results of Sag et al.[12] and our study. Both studies highlight that the accuracy of Eurofever/PRINTO criteria decreases in FMF patients with heterozygous genotype.[12,13] Similarly, in our study, we found that all criteria lost their sensitivity in heterozygous FMF patients, while the decline was the highest for clinical plus genetic Eurofever/PRINTO criteria.

A recent study from Turkey compared the efficacy of Eurofever/PRINTO criteria with the

Table 4. A summary of the studies comparing the performance of Eurofever/PRINTO criteria with other criteria

	Sensitivity/Specificity (%)				
	Gattorno et al.[11]	Sag et al.[12]	Tanatar et al.[13]*	Aydin et al.[15]	*Our study*
Eurofever/PRINTO criteria					
Clinical plus genetic	94/95	96/73.1	91.1/100	98.1/96.7	94.6/82.9
Clinical only	91/92	ND	ND	ND	93.1/80.5
Yalcinkaya Ozen criteria	ND	93.4/84.1	92.1/33.1	97.1/74.1	97.7/56.1
Tel Hashomer criteria	ND	88.7/92.6	82.6/99.2	ND	93.1/90.2
Livneh criteria	ND	ND	99.3/0.7	ND	ND

* Specificity was not specifically indicated in that study, calculated from the presented data; ND: Not determined.

Yalcinkaya-Ozen criteria in FMF patients with a single exon 10 mutation.[15] The sensitivities of these criteria were similar; however, the specificity of the new Eurofever/PRINTO classification criteria was better (96.7%) than the Yalcinkaya-Ozen criteria (76.1%), comparable to our results. The results of the relevant aforementioned studies are summarized in Table 4.

In our pediatric FMF cohort, we included patients with *M694V* homozygosity or heterozygosity. Our results confirmed that the Yalcinkaya-Ozen criteria were the most sensitive tool for FMF diagnosis; however, these criteria had a disadvantage of higher misdiagnosis rate among other AIDs. Higher sensitivity could be explained by the fact that fulfilment needs only two of its items, including fever, abdominal pain, chest pain, arthritis and family history of FMF. There are no exclusion criteria items included in the criteria; therefore, it is not surprising to have the lowest specificity. The Tel Hashomer criteria state recurrent fever, family history, and erysipelas-like erythema as minor criteria, whereas fever and accompanying symptoms are considered major criteria. Other major criteria are amyloidosis, which is rare in childhood, and colchicine response. It needs at least two major or one major plus two minor items, which makes it harder to fulfill these criteria, explaining not only an acceptable sensitivity, but also higher specificity.

In the current study, we did investigate both clinical plus genetic and clinical only Eurofever/PRINTO classification criteria in our study. We believe that necessity for two of four clinical features for diagnosis in the presence of a non-

confirmatory genotype in the clinical plus genetic criteria led to an easier diagnosis for FMF, particularly in childhood. On the other hand, clinical only criteria, which replaces the eastern Mediterranean ethnicity with the genotype, could be better to remain high sensitivity in our region; however, it could be a disadvantage for the other parts of the world. Besides, we also believe that necessity of at least six items and adding the absence of aphthous stomatitis, urticarial rash, maculopapular rash and painful lymph nodes, which are typical features of PFAPA, MKD, and TRAPS to the criteria would have better classify the patients with other AIDs. However, since a significant proportion of control patients suffered from three days of fever (43.9%) and abdominal pain (73.2%) in addition to presence of the ethnicity criteria, some of the control patients with other AIDs were still misdiagnosed as having FMF in our study.

In the light of literature and our findings, we can suggest that absence of aphthous stomatitis, urticarial or maculopapular rash and painful lymph nodes can be added to clinical plus genetic Eurofever/PRINTO classification criteria to improve its specificity. Moreover, exclusion of Mediterranean ethnicity from clinical-only Eurofever/PRINTO classification criteria would have decreased FMF diagnosis in our control patients, thereby, leading to a higher specificity. The latter maybe useful particularly in our country, where FMF is common, but other monogenic AIDs are scarce.

The main limitations of this study are its small sample size and retrospective design. The control group lacks of a higher number of patients with

AIDs, particularly MKD and TRAPS; however, being a single-center cohort makes it difficult. Further large-scale, prospective studies are needed to confirm these results.

In conclusion, the Eurofever/PRINTO classification criteria reached a comparable sensitivity for avoidance of FMF underdiagnosis in childhood. However, it is also important to distinguish other AIDs from FMF to prevent misdiagnosis and undertreatment of other AIDs. Although the Yalcinkaya-Ozen criteria have the highest sensitivity, it lack a significant specificity. Therefore, we believe that, although practitioners may benefit from all three criteria for FMF diagnosis, the Tel Hashomer criteria and Eurofever/PRINTO classification criteria have the advantage of better differentiation of FMF from other AIDs, thus, leading to less complications relevant to underdiagnosis of other AIDs.

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