A Quest to Capture Latent Fabry's Disease in a High-Risk Subpopulation of FMF.

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Research

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Abstract

Background: Familial Mediterranean fever (FMF) is an autosomal recessive disease, associated with mutations in the Mediterranean fever gene (MEFV), and manifests with recurrent episodes of febrile serositis. Fabry's disease (FD) is an X-linked lysosomal storage disease caused by mutations in the alpha-galactosidase A gene, and presents with a wide range of gastrointestinal, skin, vascular, renal and neurological manifestations. FMF and FD share similar manifestations, which may lead to misdiagnosis of one as the other; mostly FD is misdiagnosed as FMF. Moreover, various overlapping manifestations may stem from comorbidities, commonly coupled to FMF, as well as from colchicine adverse effects, which may add to the diagnostic confusion. Thus, we postulated that screening FMF for FD will lead to the identification of patients falsely diagnosed as FMF, or who suffer from FD that was previously missed.

Methods: To find missed FD among FMF population, we performed chemical and genetic analyses for FD in blood samples obtained from a cohort of FMF patients followed in the specialized FMF center of our institution. To increase the likelihood of detecting patients with FD, we enriched the surveyed FMF population with patients exhibiting manifestations shared by patients with FD or who deviate from the typical FMF presentation.

Results and conclusions: Of 172 surveyed FMF patients in a cohort derived from a clinic dedicated to FMF, none had FD. Thus, the postulation of increased odds for detecting FD in patients with FMF was not confirmed.

Background

Familial Mediterranean fever (FMF) is an autosomal recessive autoinflammatory disease, manifesting with attacks of febrile serositis and increased inflammatory markers (1). The disease is associated with mutations in the FMF gene (MEFV) that encodes pyrin (2), an inflammasomal protein, which by being mutated, predisposes the inflammasome to produce interleukin 1, the cytokine underlying FMF and driving its attacks (3). In the absence of diagnostic tests, and due to the low sensitivity and specificity of genetic testing, the diagnosis of FMF is clinical and relies on typical general and site-specific features of the attacks (4-7).

Nevertheless, a proportion of FMF patients, displaying an incomplete and atypical clinical picture, are still diagnosed with FMF, giving rise to misdiagnosis of FMF instead of another disease with comparable manifestations. Further, in addition to the typical attacks, FMF patients exhibit a wide spectrum of manifestations such as chronic abdominal, joint and muscle pain, rash, diarrhea, peripheral neuropathy, nephropathy, and others. These manifestations either stem directly from FMF, arise from comorbidities commonly occurring in FMF, or constitute adverse events of colchicine, the drug used to treat FMF (8-12). Thus, patients suffering from FMF may manifest a myriad of signs, symptoms, and laboratory findings shared by other diseases, including Fabry's disease (FD).

FD is a rare X-linked lysosomal storage disease caused by mutations in lysosomal alpha-galactosidase A (GLA), which metabolizes a sphingolipid called globotriaosylceramide (Gb3). The mutations may disrupt GLA function and lead to Gb3 deposition in various tissues, mostly blood vessels, skin, kidneys, and the central and peripheral nervous systems (13). The resulting FD phenotype is highly non-specific, comprising at least some of the following manifestations: abdominal pain, diarrhea, neuropathy, skin rash, renal failure with proteinuria, ischemic cardiovascular and central nervous system disease, fever, and more (14-17).

The diagnosis of FD usually requires a high index of suspicion in patients presenting with one or more of the nonspecific manifestations listed above, and is based on chemical and genetic analyses, demonstrating GLA enzyme inactivity and GLA gene mutation. Because of a high rate of missed and delayed diagnoses, a practice has been developed to look for patients with FD in populations with high odds to find latent FD, including in patients with undiagnosed kidney failure, young patients with cardiovascular disease, and patients with undiagnosed fever of unknown origin (18-21).

Because FMF and FD share common manifestations, FD may be misdiagnosed as FMF or may be missed as a concurrent disease in patients with FMF. Indeed, several studies reported on spotting FD among patients with FMF (22, 23). In the present study, we try to detect FD patients whose diagnosis has been missed in a cohort of FMF patients. To improve the prospects of finding FD, we pursue FD in a subpopulation of FMF enriched for manifestations shared by the two diseases.

Materials And Methods

Setting

The FMF clinic of the Chaim Sheba Medical Center at Tel Hashomer actively follows a population of about 4,000 patients. Patients are usually seen at 6-to-24-month intervals, unless urgent or more frequent visits are required. During the follow-up visits, patients' data are collected in a structured computerized file, constructed specifically for FMF patients.

Study overview

The study was carried out between December 2016 and October 2020. During this period, 500 FMF patients, who paid their regular follow-up visit to the Sheba FMF clinic, were screened by one of the authors (AL) for appropriateness for the study. Those fulfilling all study inclusion and none of the exclusion criteria, and who agreed to partake, signed an informed consent, and were enrolled in the study. Each participant was examined and interviewed and provided a blood sample to test for FD. The study was approved by the medical center's institutional review board for human experimentation.

Data collection

A questionnaire devised for the study was completed for each patient. The questionnaire collected data on FMF-associated demographic, clinical, laboratory, genetic, and treatment parameters, as well as

comorbidities and manifestations not directly related to FMF, but could potentially have stemmed from latent FD. Table 1 displays clinical features that were particularly sought to strengthen the likelihood of finding FD patients. These features were of two types: Category A – manifestations shared by FMF and FD, and Category B – features averting FMF from the typical presentation, thereby increasing the odds for a different diagnosis. Having at least one of these features was mandatory for inclusion in the study.

Inclusion criteria

A patient was enrolled in the study if meeting all the following:

1. Aged 18 years or older
2. Diagnosed with FMF based on our widely used criteria for diagnosis of FMF (24)
3. Followed in the FMF clinic repeatedly for at least five years
4. Experiencing or exhibiting one or more of the features listed in Table 1

Exclusion criteria

A patient could not be recruited to the study if meeting any of the following:

1. Unable to sign an informed consent
2. Never underwent genetic testing for FMF
3. Failed to show up for clinic follow-up visits for 5 or more years

Testing for FD

A pinprick puncture of a finger and collection of a small blood drop onto a Guthrie card was performed in the FMF clinic. Testing of the dried blood spot sample for FD was performed at Archimed laboratory (Vienna, Austria) and included analysis of enzyme activity (men) and mutational analysis of the GLA gene, using sequencing of exons and their flanking regions (women). Being a X-linked disease, men with FD display a complete lack of enzyme activity, while women may present with either partial deficiency (usually) or complete deficiency, in case the second X-chromosome is inactivated or the second GLA allele is also mutated. It was planned that both tests, genetic and enzymatic, would be performed if one of the tests was positive or borderline. In patients with borderline enzyme activity, blood Gb3 metabolites (lyso-Gb3) were tested as well (25).

Results

Patient characteristics

Of the 500 FMF patients screened, 176 fulfilled the inclusion and none of the exclusion criteria. Of these, only 172 were tested for FD (four samples failed testing for technical reasons), 60% of whom were women. Table 2 shows the main FMF features of the studied patients. As might be expected, selection

bias prompted by the inclusion criteria lessened the proportion of patients responding to colchicine to 30% and expanded the proportion of patients with various FMF-associated comorbidities to 48%, far beyond published rates in the general FMF population. Table 3 displays the actual distribution of the features specified in Table 1, favoring the capture of latent FD in the studied FMF patients. Most patients had more than one feature, bringing the number of features to a total of 333, of which 60 could be assigned to the group of manifestations of FMF shared by FD (A1), 75 to incomplete manifestations of FMF (A2), 58 to manifestations of comorbidities of FMF or of colchicine adverse effects (A3), 28 to negative genetic test for FMF (B1), and 112 to colchicine treatment failure (B2), which was the leading parameter prompting inclusion in the study.

Main findings

Of 172 FMF patients with increased odds for latent FD, none had a positive test for FD.

Discussion

In this study, we looked for latent FD in 172 FMF patients, selected because of higher risk to be misdiagnosed with FMF instead of FD, or to have FD in addition to FMF. However, despite exhibiting manifestations shared by FD, a profile different from typical FMF, or features representing both categories, none of the study patients tested positive for FD.

Our results do not concur with published reports on several FD cases that were recovered from cohorts of patients with FMF (22, 23, 26, 27). However, most of these subjects do not fit into the paradigm that FMF may mask FD. In some, the diagnosis of FMF was clearly established mistakenly, as appears for instance in a patient who received a diagnosis of FMF based on nocturnal fever lasting several months, bilateral finger arthritis, and carriage of a non-pathogenic MEFV mutation (22). Others, either did not display manifestations of FD or had only a few mild symptoms, such as occasional paresthesia or mild “cardiac symptoms” (22), which, independent of FMF, will not elicit exploration for FD, and therefore the diagnosis of FD was not actually missed due to concordant FMF. In the remaining patients, the data provided is insufficient to determine whether they should have received a diagnosis of FMF or FD to begin with (22, 23).

There have been at least two published cases in which FD was identified in patients who fulfilled criteria for FMF (26, 27). Per the data available, the diagnosis of FMF rested on both recurrent short episodes of febrile abdominal attacks and FMF genetic testing. Later, FD was diagnosed in one case based on kidney biopsy performed for proteinuria, and then on GLA activity, which was nil; and in the other case, on clinical, chemistry and genetic analysis for FD. Thus, concealing of FD by FMF does occur, and contradicts our findings.

Three possible explanations might be applied to our nonsuccess to detect even one case:

1. *Sample size* – The prevalence of FD in the general population ranges between 1:100,000 to 1: 22,000 (13, 28). Therefore, to find even one case of FD among the 172 patients studied, FMF must enrich the odds by a factor of 128-580, which is clearly much more than expected from FMF.
2. *Setting* – In an FMF-dedicated clinic, misdiagnosis of FD as FMF is highly unlikely, as for experts the diagnosis of FMF is based on deep recognition of the disease and its fine features.
3. *Comorbidities of FMF* – Although FD might be masked more readily in FMF patients affected by additional inflammatory disorders, most FMF patients with inflammatory comorbidity typically suffer from characteristic FMF and therefore are less likely to be misdiagnosed.

In conclusion, in a study of 172 FMF patients selected by virtue of increased risk of including FD patients misdiagnosed as FMF, or with both FMF and overlooked FD, we failed to detect even a single patient with FD. This may result from the strategy of the study, though it more likely it reflects the high standard of FMF diagnosis found in an FMF-dedicated clinic.

Abbreviations

CVA – Cerebrovascular accident, FD – Fabry's disease, FMF – Familial Mediterranean fever, Gb3 - globotriaosylceramide, GLA - alpha-galactosidase A, MEFV – Mediterranean fever gene, MI – Myocardial infarction, TIA – Transient ischemic attack, US – Ultrasound,

Declarations

Ethics approval and consent to participate - Approval from the Sheba Medical Center Ethics Committee was obtained. Signed informed consents were obtained from all participants.

Consent for publication - No individual data that require consent for publication are published

Availability of data and materials - The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests - All authors declare no competing interests.

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Authors' contributions – All authors contributed substantially to the analysis, interpretation of data, writing and revision of the manuscript. In addition, AL devised and designed the study and AL and TM acquired the dataset

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Tables

Table 1. Features increasing likelihood of spotting latent or misdiagnosed FD among FMF patients

Category	Group	Manifestation / feature	Definition of the manifestation / feature
A. Overlapping manifestations	A1. Typical manifestations of FMF	Proteinuria or kidney function impairment	Of unknown cause. Amyloid nephropathy was excluded by biopsy, or atypical clinical course*
		Splenomegaly	Long axis is more than 13 cm by US imaging
		Recurrent episodes with fever alone	Unexplained fever episodes, not accompanied by abdominal, chest or joint pain, may be accompanied by constitutional manifestations**
	A2. Incomplete manifestations of FMF	Abdominal pain	Afebrile attacks, localized rather than diffused pain, longer than a week
		Joint involvement	Arthralgia, afebrile episodes, long duration (>week)
	A3. Manifestations of FMF comorbidities or colchicine adverse effects	Ischemic cardiovascular disease	MI, TIA, CVA in patients younger than 50 years
		Fibromyalgia - like pain	Particularly limb pain and generalized pain, without explanation, therefore labelled fibromyalgia
		Neuropathic pain	Including numbness paresthesia, burning pain without explanation
B. Features diverting the FMF from typical presentation	B1. Genetic testing	Genetic negative FMF	Absence of at least 5 most common MEFV mutations (M694V, V726A, E148Q, M680I, M694I)
	B2. Treatment experience	Colchicine failure	Lack of FMF response to at least 2 mg/day of colchicine. Intolerance or allergy to colchicine are excluded.

*Typical course of amyloid nephropathy – Slowly growing proteinuria, usually reaching a nephrotic stage, followed by slow evolution of renal function loss; **Constitutional manifestations – Chills, diffuse muscle pain, headache, tiredness, weakness, sweating. Abbreviations: US – Ultrasound, CVA – Cerebrovascular

accident, FMF – Familial Mediterranean fever, FD – Fabry’s disease, MEFV – Mediterranean fever gene, MI – Myocardial infarction, TIA – Transient ischemic attack

Table 2. FMF features in a cohort of FMF patients evaluated for FD manifestations.

Set	Feature	Finding
Demographic traits	Age at study entry - years, mean $\pm$ SD	47 $\pm$ 14
	Age at FMF onset - years, mean $\pm$ SD	13 $\pm$ 12
	Female sex - n, (%)	104, (60)
	Family history of FMF - n, (%)	128, (74)
Manifestations	Abdominal attacks - n, (%)	163, (95)
	Chest attacks - n, (%)	109, (63)
	Arthritis attacks - n, (%)	107, (62)
	Erysipeloid rash - n, (%)	17, (10)
	Acute scrotum - n, (%)	5, (3)
	Short-term muscle attacks - n, (%)	1, (1)
	Long-term muscle attacks - n, (%)	3, (2)
	Fever only attacks - n, (%)	12, (7)
	Leg pain - n, (%)	115, (67)
Associated comorbidities	Inflammatory bowel disease - n, (%)	3, (2)
	Ankylosing spondylitis - n, (%)	29, (17)
	Behçet’s disease - n, (%)	48, (28)
	Fibromyalgia - n, (%)	26, (15)
	Patients with any comorbidity - n, (%)	82, (48)
Treatment	Colchicine - n, (%)	164, (95)
	Mean colchicine dose - mg/day, mean $\pm$ SD	1.9 $\pm$ 0.84
	Colchicine dose $\geq$ 2 mg/day - n, (%)	103, (60)
	Response to colchicine - n, (%)	52, (30)
	Biologic drugs - n, (%)	15, (9)
Genetics	Two or more mutations - n, (%)	98, (57)
	One mutation - n, (%)	35, (20)
	No mutations in genetic testing - n, (%)	28, (16)
	M694V / M694V - n, (%)	65, (38)
	M694V / 0 - n, (%)	28, (16)

Table 3. Distribution in the study group of features favoring detection of FD

Manifestation / feature	Group	Number (%)
Proteinuria	A1	27 (16)
Renal failure	A1	5 (3)
Spleen enlargement	A1	16 (9)
Fever only	A1	12 (7)
Incomplete abdominal attacks	A2	16 (9)
Joint involvements which are not typical of FMF	A2	59 (34)
Fibromyalgia-like manifestations	A3	1 (1)
Neuropathy	A3	24 (14)
Cardiomyopathy / atherosclerotic heart disease	A3	14 (8)
Peripheral vascular disease	A3	5 (3)
CNS vascular disease	A3	14 (8)
Absence of mutations in genetic testing	B1	28 (16)
Lack of response to colchicine	B2	112 (65)