UDC 616.155.392
DOI: 10.54503/0514-7484-2026-66.2-83

# Paroxysmal Nocturnal Hemoglobinuria in an Adolescent with MEFV Variant and Thrombotic Risk Factors: Case Report Including a TTP-Like Life-Threatening Event and Subsequent Response to Eculizumab

**H.S. Khachatryan[1,2], L. Luzzatto[3,4], W. Barcellini [5], E.A. Avetisyan[2], A.S. Stepanyan[2], H.Sh. Sayiyan[2], A.A. Voskanyan[1], G.N. Tamamyan[2], H.G. Grigoryan[1], L.S. Sahakyan[1], A.H. Zakharyan[1,2], N.S. Sargsyan[1,2]**

[1]*Yeolyan Hematology and Oncology Center*
*Yerevan, 0014, 7 Nersisyan St.*
[2]*Yerevan State Medical University after M.Heratsi*
*Yerevan, 0025, 2 Koryun St.*
[3]*University of Florence*
*50121, Florence, Italy 4 P.za di San Marco, Firenze FI*
[4]*Muhimbili University of Health and Allied Sciences*
*P.O. Box 65001, United Nations Road,*
*Dar es Salaam, Tanzania*
[5]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, University of Milan*
*Via Francesco Sforza 28,*
*20122, Milan, Italy*

*Keywords:* paroxysmal nocturnal hemoglobinuria (PNH), complement-mediated hemolysis, PIGA gene mutation, glycosylphosphatidylinositol (GPI) anchor deficiency, intravascular hemolysis, thrombosis risk, eculizumab treatment

## Introduction

In paroxysmal nocturnal hemoglobinuria (PNH) one or more clones of blood cells develops from stem cells that have an acquired mutation in the X-linked *PIGA* gene [6]. The *PIGA* gene encodes phosphatidylinositol glycan complementation class A, an enzyme that catalyses an early and essential step in glycosylphosphatidylinositol (GPI) anchor synthesis. Thus cells are deficient in all GPI anchored proteins, including CD55 and CD59 which regulate complement activation. PNH usually develops in patients with aplastic anemia (AA) and it is thought that PNH cells have a growth or survival advantage over the AA cells although the mechanism is not known [3]. PNH cells can be completely deficient in GPI anchored proteins (Type III) or partially deficient due to residual activity of the PIGA protein (Type II), while PNH Type I cells express GPI-linked proteins normally.

Clinically, PNH is characterized by bone marrow failure, thrombosis and intravascular hemolysis. Recently the use of a complement inhibitor, eculizumab has greatly improved the quality of life of PNH patients as it causes a dramatic reduction in the hemolysis and thrombotic episodes, improvement in anemia, with a stabilization of the hemoglobin levels and reduced transfusion requirements [2]. Eculizumab leads to an increase in the number of circulating red blood cells that otherwise are subject to complement-mediated hemolysis [1].

Familial Mediterranean fever (FMF) is an autosomal recessive condition (MIM 249100) that primarily affects populations surrounding the Mediterranean basin, the disease being restricted essentially to Armenian, Sephardic Jewish, Turkish, and Arab populations [8]. The frequency of heterozygotes, as deduced from the prevalence of the disease, is extremely high in those populations, reaching 1:7 among Armenians [7]. This disease is characterized by recurrent episodes of fever and serosal inflammation manifested by sterile peritonitis, arthritis, and/or pleurisy, sometimes associated with erysipelas-like erythema. The major complication of FMF is amyloidosis, mainly renal, which develops over years and progresses to terminal renal failure [8].

Given the absence of pathognomonic clinical symptoms and of any specific biochemical abnormality, the diagnosis of FMF is, at present, one of exclusion; it can be made only retrospectively, and is based entirely on clinical criteria [5]. It is, however, of prime importance to ascertain this diagnosis, for the following reasons. First, the symptomatology of FMF may mimic that of other affections—such as acute peritonitis, appendicitis, cholecystitis, or arthritis—thereby leading to unnecessary exploratory surgery [8]. Second, an effective therapy is available: daily and lifelong administration of colchicine not only reduces the frequency and severity of attacks [4, 9] but also prevents amyloidosis and transplantation for renal failure [10].

Thrombophilia is any violation of the physiologically equilibrium state of the hemostasis system, leading to an increased tendency of the body to thrombus formation. The main clinical manifestations of this condition are deep vein thrombosis of the lower extremities (DVT) and pulmonary embolism (PE), characterized by the ability of a thrombus to migrate with the bloodstream and a high probability of blockage of coronary arteries. The consequences of thrombophilia are such serious complications as ischemic heart and brain diseases, including myocardial infarction and stroke, as well as other cardiovascular diseases. Genetically predetermined thrombophilia is a consequence of genetic mutations inherited from parents and causing a hereditary predisposition of the body to the development of thrombosis. It should be noted that hereditary predisposition is not always, and in some cases throughout life, may not be complicated by thrombotic manifestations. However, as a result of the "provoking" action of various "external factors", the risk of thrombosis in individuals with hereditary thrombophilia increases significantly. These triggers include pregnancy, oral contraceptive use, and hormone replacement therapy. According to modern

concepts, the occurrence of thrombosis requires the interaction of a number of factors, both hereditary and acquired. It has been shown that the carriage of certain genetic mutations in the blood coagulation and fibrinolysis system is one of the unfavorable factors that significantly increase the risk of thrombosis. The identification of such mutations helps to identify the risk group at an early stage and make appropriate adjustments to the management of these patients.

## Materials and Methods

Peripheral blood from patient was obtained after getting written informed consent. Blood samples for fluorescent cytometry and electrophoretic analyses were obtained from EDTA tubes. Flow cytometry studies carried out in two independent laboratories (one in St. Petersburg and one in Budapest Semmelweis University) have found a population of GPI-negative cells of about 87% in granulocytes and about 91% in monocytes. Mononuclear cells isolated from peripheral blood were investigated. 100.000 hits were collected and investigated for the markers of the PNH panel: FLAER/CD15/CD45/CD64/CD157.

The implications, if any, of PNH having developed in a heterozygote for a mutation of the gene MEFV encoding pyrin are not known (I believe this situation is without precedents).

Genomic DNA was isolated from peripheral leukocytes, by standard procedures. Different methods were used to screen for MEFV mutations

PCR primers to detect mutations confirming the MEFV genotype were designed with the Center of Genetics of Armenia.

The MEFV genotype of each affected individual carrying at least two different mutations was accurately identified by different means, depending on both the availability of parental DNA samples and the location and nature of the two MEFV mutations.

When considering the primary diagnostic concept, a family history of a periodic fever disease was taken into account (the mother suffers from Periodic fever disease, the diagnosis is confirmed, she constantly takes Colchicine, the child has a heterozygous mutation V726A of the MEFV gene), as well as an indication of the possibility of tropical malaria (possible infection in Uganda, 4 children got sick during the group excursions). During the examination, the diagnosis of malaria was not confirmed in the laboratory, but the treatment was carried out.

A thrombophilia tests were performed by PCR, all coagulation panel screen has yielded PT, APTT, D-dimer; protein S,AT III; Factor V activity; protein C, homocysteine.

Testing for genetic mutations (Gen-diagnostics) is based on use of highly specific PCR technology. The reliability of the results of genetic testing does not depend on the indicators of other diagnostic tests and on the patient's condition. Genetic testing can be performed at any stage of patient observation and treatment. PCR technologies used in the laboratory: PCR with electrophoretic detection, PCR

with subsequent restriction of amplicons - "RFLP" technology, PCR with subsequent hybridization - "BioChip" technology, real-time PCR - "Bio-Rad" technology.

Primary remote consultation according to the history of the child's parents, the medical documentation provided and the results of the study of blood samples delivered to the 3 centers:

School of Medicine department of Haematology and Blood Transfusion Muhimbili University of Health and Allied Sciences, Tanzania, University of London, Scientific Research Institute NII DOG and R. M. Garbachev PSPOGMU after I.P. Pavlova.

The purpose of the consultation: to clarify the diagnosis and determine treatment tactics. The anamnesis is examined in detail and the examinations performed at previous stages in Uganda and Republic of Armenia.

## Case report

Patient S. M. born on 11.11.2004; this 15 year old boy came to the Hematology Clinic, accompanied by his mother, for a consultation about management of paroxysmal nocturnal hemoglobinuria (PNH), after extensive correspondence we had had previously.

He is the second of his parents' two sons; he was healthy until the age of 12: a blood count that I saw, carried out in 2011, was entirely normal. In 2017 when the boy had some abdominal complaint, the endoscopy revealed 'duodenitis' inflammation; he was not treated for H pylori, although one test was positive.

In 2019, the boy noted that occasionally his urine was dark, he could recall 5 such cases, lasting from hours to 2–3 days; he could not say whether these episodes were triggered by something. He also may have had malaria at least once, for which he was treated. A blood test revealed anemia, and subsequent extensive investigations led to the diagnosis of PNH.

In the family history, mother has been diagnosed with Familial Mediterranean Fever (FMF), having bi-allelic MEFV mutations (M694V/V726A); patient is heterozygous for the V726A allele.

Patient is a well developed young man who appears somewhat pale and a bit jaundiced, but otherwise well. He reported having had transient dysphagia recently. There are no peripheral lymph nodes, no peripheral edema. Apart from slightly inflamed oro-pharynx, systemic review was essentially negative; in particular, I could not feel spleen or liver, even in deep inspiration.

From review of previous laboratory results (from various different labs over the past several months), the Hb has ranged from 7.1 to 9.5 (G/dl); the absolute reticulocyte count from 92 to 228 ($x10^3$/Ul); the absolute neutrophil count (ANC) from 1.0 to 7.4 ($x10^9$/l); the platelets from 98 to 155 ($x10^9$/l); LDH from 1160 to 2042, bilirubin 19.5 (unconjugated 15.5); creatinine 25.

A thrombophilia screen has yielded normal PT and APTT; normal D-dimer; protein S 85%; AT III 107%; Factor V activity 68%; protein C 59%. A report from another laboratory shows elevated homocysteine.

Flow cytometry studies carried out in two independent laboratories (one in St. Petersburg and one in Budapest) have found a population of GPI-negative cells of about 87% in granulocytes and about 91% in monocytes.

Results of a blood count carried out here (attached) are in keeping with previous records. The peripheral blood smear showed considerable anisocytosis, microcytes, poikilocytes, target cells, hypochromic cells: in keeping with high RDW and low MCHC, this indicates iron deficiency. The total bilirubin was 45 (unconjugated 33); LDH 4500: these data indicate both intravascular and extravascular hemolysis. A Ham test was positive (++). Bone marrow aspiration was not done because it has been done previously and reported as adequate (I have not seen the report yet).

On grounds of all of the above, the diagnosis of PNH must be regarded as established. I have discussed the diagnosis and several aspects of this condition with the boy and his mother, particularly with respect to management.

Considering moderate to severe anemia, significant blood transfusion history and size of the PNH cell population, there is a clear indication for starting the patient on eculizumab (or on rovelizumab). Unfortunately, this is not available in either Uganda or Armenia; therefore we had to focus, for the moment, on a management plan without these agents. In this light, we are listing here considerations/recommendations.

Since the initial assessment, eculizumab has become available for this patient and was commenced approximately five months prior to this report, following consultation with Professor Lucio Luzzatto and with ongoing specialist follow-up by Dr Wilma Barcellini (Milan). The introduction of eculizumab has been clinically advantageous, with an overall improvement in disease control consistent with reduced complement-mediated hemolysis and thrombotic risk.

1. The boy has a reasonably good quality of life and that should continue. He can live with PNH and he should not feel excessively medicalized. However, PNH can be a serious disorder; therefore his lifestyle should avoid, as far as possible, stressful or excessive situations. I got the impression that, fortunately, he is a sensible person and he will be able to reconcile activity with moderation.

2. The blood results show iron deficiency: this is not unusual in PNH, as much iron is lost from the body with hemoglobinuria. Patient is already on oral iron that should be continued (FeSO4 200 mg/day) until the iron deficiency is corrected. In view of increased demand by the bone marrow, he should also continue folic acid (5 mg/day).

3. PNH carries a significant risk of venous thrombosis (the risk would be significantly decreased, though not eliminated, if the patient were on eculizumab).

Fortunately patient gives no history suggestive of any previous episode of thrombosis: however, he does have a partial deficiency of protein C and an

elevated serum homocysteine level, both of which may indicate an increased risk of thrombosis. Therefore, in our view the boy ought to start anticoagulant prophylaxis. Conventionally this is done by using subcutaneous heparin for a few days, overlapping with and followed by oral warfarin, aiming for INR to be always between 2.5 and 3.5. This treatment must be supervised by a physician familiar with it; and it will require initially weekly INR monitoring; once dose/level are stable, monthly monitoring may be sufficient. Having consulted with Professor Lucio Luzzatto (School of Medicine, Department of Haematology and Blood Transfusion, Muhimbili University of Health and Allied Sciences, Tanzania) and Professor M Laffan of University of London, with Professor Kulagin A.D. (PSP6GMU after Acad. I.P. Pavlov on Hematology).

We think one might consider, as an alternative, apixaban (2.5 mg every 12 hours): this has the considerable advantage that INR monitoring is not required. On the other hand, although this agent is highly efficacious, there is no record, as yet, on its efficacy in preventing thrombosis in PNH specifically.

4. As long as patient lives in a malaria-endemic area, he should be, on anti-malarial prophylaxis. Approved alternative regimens are mefloquine, doxycycline, atovaquone/proguanil. Since each one of these drugs may entail side effects (including their price), a possible alternative is proguanil (= paludrine) alone, 100 mg/day: this may not give 100% protection, but it is far better than having no protection at all. Proguanil should be taken every day while in a malaria-endemic area, and for 14 more days after leaving.

5. Microscopic hemoglobinuria is probably present in patient’s urine all the time; but when it becomes macroscopic – i.e. obvious to the eye – it is a warning that hemolysis has increased: a doctor should be informed and a blood count should be obtained promptly.

6. The implications, if any, of PNH having developed in a heterozygote for a mutation of the gene MEFV encoding pyrin are not known (I believe this situation is without precedents). Potentially this combination may be associated with increased tendency to inflammatory symptoms, and it may explain why in July 2019 patient’s ESR was persistently somewhat elevated. We don’t think there is any indication for prolonged use of colchicine, but perhaps a short use may be considered in certain circumstances.

7. We have discussed bone marrow transplantation (BMT). Since eculizumab has been introduced BMT is used less, but it is still the only curative therapy for PNH. I would recommend HLA typing on the patient and brother (an HLA-identical sib donor is still the preferable donor, even though alternatives are possible).

## Results and Discussion

The main diagnosis: Paroxysmal nocturnal hemoglobinuria, classic form D59.5 (debut 2014?, verification on 06.08.2019). Chronic intravascular hemolysis.

Hemolytic crises. Anemia III( with the need for transfusions of red blood cells). Abdominal syndrome.

The diagnosis of PNH is not doubt, fully documented by the results of the examinations. Bone marrow failure is not expressed, there are no peripheral criteria in favor of aplastic anemia. A full examination is planned for the severity of bone marrow failure.

The patient belongs to a high-risk group for further progression of PNH, the development of life-threatening thrombotic and progression of organ complications against the background of uncontrolled intravascular hemolysis. Current clinical status (the course of active hemolytic PNH with deep anemia and the need for red blood cell transfusions) according to current International and National recommendations (2014) requires the appointment of targeted anticomplementary therapy. The boy's parents are invited to consider participating in a clinical trial of ravulizumab (ALXN1210).

According to the results of the examination and dynamic observation against the background of anticomplementary therapy, therapeutic tactics can be adjusted, including considering the indications and the possibility of allogeneic hematopoietic stem cell transplantation. It is planned to carry out HLA-genotyping of the patient and potential donor KM (sibling).

Recommended:

Observation of a hematologist, consultation of a nephrologist. Careful monitoring of clinical manifestations of intravascular hemolysis, manifestations of nephropathy, high clinical alertness in relation to thrombotic complications.

Routine laboratory control: a clinical blood test with reticulocytes, a clinical analysis of urine, lactate dehydrogenase, bilirubin fractions, creatinine once in 2 weeks.

Control the size of the APG clone once every 6-12 months.

Research (trepan biopsy, bone marrow hematopoiesis myelogram, cytogenetic study, colony forming ability) in a planned manner. HLA-genotyping of the patient and potential related donors( brother).

The patient is shown continuous anti-complementary therapy (eculizumab). An alternative option is therapy with Rovelizumab (ALXN1210) as part of a clinical study.

Prior to the initiation of anti-complementary therapy, vaccination against meningococcus with a tetravalent vaccine (Menactra), pneumococcus, and hemophilic bacillus is indicated.

Folic acid 5 mg/day continuously.

Determination of the concentration of ferritin once every 6 months, with documentation of deficiency, treatment with iron preparations to control the intensity of hemolysis.

## Patient consent

Written informed consent was obtained from the patient for publication of their clinical details.

## Author contributions

Author conceived the study, NP designed the experiments and carried out the research. MM collected and collated clinical data. All authors contributed to preparing a draft of the manuscript and have agreed to the final content.'

*Accepted 10.01.26*

## Пароксизмальная ночная гемоглобинурия у подростка с вариантом гена MEFV и факторами тромботического риска: клинический случай с ТТП-подобным жизнеугрожающим эпизодом и последующим ответом на терапию экулизумабом

**Е.С. Хачатрян, Л. Луццатто, В. Барчеллини, Э.А. Аветисян, А.С. Степанян, Э.Ш. Саиян , А.А. Восканян, Г.Н. Тамамян, А.Г. Григорян, Л.С. Саакян, А.А. Захарян, Н.С. Саргсян**

Мы представляем сложный клинический случай пароксизмальной ночной гемоглобинурии (ПНГ), характеризующийся сочетанием нескольких гематологических и генетических факторов, включая гетерозиготный вариант гена MEFV, дефицит естественных антикоагулянтов, гипергомоцистеинемию, эпизод малярийной инфекции и анемический синдром. Ведение пациента осуществлялось при участии специалистов из Армении, России, Танзании и Великобритании.

Для армянской медицинской аудитории описанный случай представляет особый интерес как первое детально исследованное наблюдение подобного рода, представленное в национальной научной литературе. Вместе с тем для международного сообщества, учитывая значительный объём публикаций по ПНГ, ключевым является выделение потенциально новых научных аспектов представленного наблюдения.

Первым значимым аспектом является развитие в июне 2020 года жизнеугрожающего состояния, сопровождавшегося выраженной тромбоцитопенией (<5×10⁹/л) и клинической картиной, высоко подозрительной на тромботическую тромбоцитопеническую пурпуру (ТТП). Ведение этого эпизода осуществлялось при участии Лючио Луццатто совместно с коллегами из Уганды; пациенту была проведена терапия глюкокортикостероидами и плазмообмен. Последующие лабораторные исследования, выполненные в Милане, поддержали этот диагноз. По имеющимся сведениям, это может представлять собой один из первых описан-

ных случаев ТТП у пациента с ПНГ. Однако, учитывая неполноту диагностического набора (в частности, отсутствие полной верификации), указанный эпизод следует рассматривать как вероятный ТТП или ТТП-подобный синдром.

Вторым важным аспектом является наличие гетерозиготного варианта гена MEFV. Несмотря на отсутствие клинических критериев семейной средиземноморской лихорадки, повторяющиеся эпизоды лихорадки и повышение уровня С-реактивного белка могут указывать на возможное патофизиологическое взаимодействие между ПНГ и носительством этой мутации.

Следует также отметить, что терапия ингибитором комплемента экулизумабом была начата под руководством Лючио Луццатто и впоследствии Вильмы Барчеллини (Милан) и сопровождалась выраженным клиническим улучшением.

## Պարոքսիզմալ գիշերային հեմոգլոբինուրիա դեռահասի մոտ՝ **MEFV** գենի տարբերակի և թրոմբոզի ռիսկի գործոնների համակցությամբ. դեպքի նկարագրություն՝ **TTP**-ին նման կյանքին սպառնացող դրվագով և էկուլիզումաբով արդյունավետ բուժմամբ

**Հ.Ս. Խաչատրյան, Լ. Լուցցատտո, Վ. Բարչելլինի, Է.Ա. Ավետիսյան, Ա.Ս. Ստեփանյան, Հ.Շ. Սայիյան, Ա.Ա. Ոսկանյան, Գ.Ն. Թամամյան, Հ.Գ. Գրիգորյան, Լ.Ս. Սահակյան, Ա.Հ. Զախարյան, Ն.Ս. Սարգսյան**

Ներկայացնում ենք պարոքսիզմալ գիշերային հեմոգլոբինուրիայի (PNH) կլինիկական դեպք:

Դեպքն առանձնանում է իր բազմաբաղադրիչ և յուրահատուկ կլինիկական ընթացքով: Այն համակցվում է Միջերկրածովյան ընտանեկան հիվանդության (MEFV) գենի մուտացիայի առկայությամբ, բնական հակամակարդիչների դեֆիցիտով, հիպերհոմոցիստեինեմիայով, մալարիայի էպիզոդիկ վարակով և անեմիայով: Այս կլինիկական դեպքը վերահսկվել է փորձառու մասնագետների կողմից՝ Հայաստանի, Ռուսաստանի, Տանզանիայի և Անգլիայի:

Հայկական բժշկական հանրության համար սա առաջին դեպքն է, որն ուսումնասիրվել է այսպիսի խորությամբ, ուստի նույնիսկ կարճ դեպքի ներկայացումը կարող է հետաքրքրություն առաջացնել տեղական բժշկական ամսագրում:

Միջազգային ամսագրի ընթերցողների համար PNH-ի վերաբերյալ գրականությունն արդեն բավական ընդարձակ է, հետևաբար՝ կարևոր է ընդգծել հնարավոր նոր գիտական ուղերձը: Մեր գնահատմամբ առկա են առնվազն երկու նշանակալի դիտարկում:

Հիվանդի անամնեզի համաձայն՝ 2020 թ. հունիսին տղայի մոտ զարգացել է կյանքին սպառնացող բարդություն՝ արտահայտված թրոմբոցիտոպե-

նիայով (թրոմբոցիտներ <5000/մկլ)։ Իտալացի բժիշկ պրոֆեսոր Լ. Լուցցատտոյի և ուգանդացի գործընկերների մասնակցությամբ էպիզոդը բուժվել է կորտիկոստերոիդներով և թերապևտիկ պլազմափոխանակմամբ՝ TTP-ի կասկածով։ Հետագայում Միլանում իրականացված հետազոտությունների հիման վրա ստացված եզրակացությունը հաստատել է այս ախտորոշումը։ Մեր տեղեկություններով սա PNH ունեցող հիվանդի մոտ TTP-ի առաջին նկարագրված դեպքն է։

Երկրորդ կարևոր հանգամանքը MEFV գենի մուտացիայի համակցված առկայությունն է։ Թեև հիվանդը չունի ընտանեկան միջերկրածովյան տենդի (FMF) դասական կլինիկական պատկեր, չի բացառվում, որ հաճախակի տենդային էպիզոդներն ու բարձր CRP-ն պայմանավորված լինեն PNH-ի և MEFV հետերոզիգոտ մուտացիայի հնարավոր փոխազդեցությամբ։

2020 թ. հունիսին հիվանդի մոտ արձանագրվել է կյանքին սպառնացող վիճակ՝ խորը թրոմբոցիտոպենիայով (<5×10⁹/Լ) և TTP-ին բնորոշ կլինիկական պատկերով։ Էպիզոդը բուժվել է կորտիկոստերոիդներով և թերապևտիկ պլազմափոխանակմամբ, ինչից հետո նկատվել է կլինիկական կայունացում։ Թեև առկա տվյալներն աջակցում են PNH-ի ֆոնին TTP-ի աշխատանքային ախտորոշմանը՝ համակցություն, որը նախկինում չի նկարագրվել, ախտորոշիչ տեղեկատվությունը դեռ ամբողջական չէ։ Ուստի այս դրվագը ներկայացվում է որպես հավանական TTP կամ TTP-նման էպիզոդ մինչև ամբողջական փաստաթղթավորման ստացումը։

Կարևոր է նշել, որ կոմպլեմենտի ինհիբիցիան էկուլիզումաբով սկսվել է ձեռագրի պատրաստումից մոտ հինգ ամիս առաջ պրոֆեսոր Լուչիո Լուցցատտոյի և հետո դոկտոր Վիլմա Բարչելլինիի (Միլան) հսկողությամբ և ուղեկցվել է ակնհայտ բարենպաստ կլինիկական ընթացքով։

## References


1. *Borowitz M. J. et al.,* “Guidelines for the Diagnosis and Monitoring of Paroxysmal Nocturnal Hemoglobinuria and Related Disorders by Flow Cytometry,” vol. 230, no. April, pp. 211–230, 2010, doi: 10.1002/cyto.b.20525.
2. *Brando B., A. Gatti, and F. Preijers,* “Flow cytometric diagnosis of paroxysmal nocturnal hemoglobinuria : pearls and pitfalls – a critical review article,” vol. 30, pp. 355–370, 2019.
3. *Brodsky R. A.,* “Paroxysmal nocturnal hemoglobinuria,” vol. 124, no. 18, pp. 2804–2812, 2014, doi: 10.1182/blood-2014-02-522128.2804.
4. *Dinarello C. A., S. M. Wolff, S. E. Goldfinger, D. C. Dale, and D. W. Alling,* “Colchicine therapy for familial mediterranean fever. A double-blind trial,” N. Engl. J. Med., vol. 291, no. 18, pp. 934–937, 1974.
5. *Livneh A. et al.,* “Criteria for the diagnosis of familial Mediterranean fever,” Arthritis Rheum., vol. 40, no. 10, pp. 1879–1885, 1997.

6. *Parker C. et al.,* “Review in translational hematology Diagnosis and management of paroxysmal nocturnal hemoglobinuria,” vol. 106, no. 12, pp. 3699–3709, 2005, doi: 10.1182/blood-2005-04-1717.Supported.
7. *Rogers J. I., Shohat D. B., Petersen M., Bickal G. M., Congleton J., Schwabe J., Rotter A. D.,,* “Familial Mediterranean fever in Armenians: autosomal
8. *Sohar H., Gafn E., Pras J., Heller M.,* “Familial Mediterranean fever. A survey of 470 cases and review of the literature.,” Am. J. Med. (Am J Med), vol. 43, pp. 227–253, 1967.
9. *Zemer D. et al.,* “A controlled trial of colchicine in preventing attacks of familial mediterranean fever,” N. Engl. J. Med., vol. 291, no. 18, pp. 932–934, 1974.
10. *Zemer D. Zemer, Pras M.,Sohar E., Modan M.,Cabili S., and Gafn J.,* “Colchicine in the prevention and treatment of the amyloidosis of familial Mediterranean fever,” N. Engl. J. Med., vol. 314, no. 16, pp. 1001–1005, 1986.