

Paroxysmal Cold Hemoglobinuria with Positive Treponema Immobilization Test. Report of Case

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PAROXYSMAL COLD HEMOGLOBINURIA (PCH), originally described by Dressler¹ a hundred years ago, is characterized by a positive Wassermann reaction, a positive Donath-Landsteiner test and a low cold agglutinin titer. A second form reported by Salén² is associated with a negative Wassermann reaction, a negative Donath-Landsteiner reaction and a high cold agglutinin titer.^{3, 4} The etiologic role of syphilis has been a matter of debate for as long as serologic tests for syphilis have been applied to patients with paroxysmal cold hemoglobinuria. The treponema immobilization test (TPI) of Nelson and Mayer should contribute to the solution of this question.

Case History

M. V., a housewife, 34 years old reported that her father was said to have had malaria during World War I. The patient's mother was likewise believed to have had malaria while pregnant. According to a relative the patient was a feeble infant, frequently ill with fever and the recipient of anti-malarial therapy. The family denied history of syphilis or PCH.

During childhood the patient had measles, whooping cough, and chickenpox. At age seven she was hospitalized because of a disease of the knee joints. In her 15th and 16th year she was treated for malaria with quinine.

During the past 5 to 6 years when working in the cold the patient would become weak and pale. In the winter of 1952 when exposed to cold she would develop fever, chills, headache, nausea and lumbar pain. The patient noticed that at these times her urine was dark, describing it "like iodine."

At present the patient's appetite is good, stools are regular, as are her menstrual periods. She has a slight nocturnal frequency and has borne a full-term, healthy child. She denies venereal infection.

Physical Examination revealed a tall, well nourished woman whose skin was pale-yellowish. The pupils were equal and regular, reacting normally to light, accommodation, and convergence. The mouth, teeth and tonsils were not remarkable.

The lungs were clear to auscultation and percussion. The heart outline and sounds were normal. Blood pressure was 180/85 mm. of Hg and pulse rate was 80 per minute.

The liver was one finger breadth below the costal margin and the spleen was palpable.

External genitalia were normal as were the extremities. The reflexes were not unusual. The patient's gait was normal as were motor and sensibility tests.

Laboratory Findings: Sedimentation rate (Westergren) was 68 mm., corrected to 31 mm. Erythrocytes were 2,800,000 per cu. mm., hemoglobin was 59% (9.4 Gm.), the color index was 1, leukocytes were 4,600 per cu. mm., and the hemogram revealed eosinophilic leukocytes 3%, neutrophilic polymorphonuclear leukocytes 69% of which stab forms were 5%, and monocytes 5%. The hematocrit was 31%, MCV 97 cu. μ , MCH 32 and MCHC 34%. Microscopic examination of a smear preparation showed anisocytosis, macrocytosis, poikilocytosis and polychromasia of the erythrocytes.

The specific gravity of the urine was 1018 and tests for albumin, sugar, bilirubin and blood were all negative. The urobilinogen was 1:16. Urinary sediment was not remarkable, and dilution and concentration tests showed values within normal limits.

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TABLE 1.—Quantity of Hemoglobin in Blood and Urine in Rosenbach Test

| Date of Test | Blood | | | Urine | | |
|----------------|--|-------------------------------|------------------------|--|-------------------------------|------------------------|
| | Quantity of hemoglobin before exposure to cold | During attack | | Quantity of hemoglobin before exposure to cold | During attack | |
| | | Time from beginning of attack | Quantity of hemoglobin | | Time from beginning of attack | Quantity of hemoglobin |
| Dec. 27, 1952 | φ | 30 min. | 0.169 Gm. % | φ | 1 hr. | 0.327 Gm. % |
| | | | | | 3 hr. | φ |
| | | | | | 6 hr. | φ |
| March 16, 1953 | φ | 25 min. | 0.10 Gm. % | φ | 50 min. | 3.43 Gm. % |
| | | | | | 2 hr. | 0.15 Gm. % |
| | | | | | 3 hr. | φ |
| | | | | | 6 hr. | φ |
| May 14, 1953 | φ | 30 min. | 0.20 Gm. % | φ | 1 hr. | 1.92 Gm. % |
| | | | | | 1 hr. 30 min. | 1.05 Gm. % |

The test of December 27, 1952, was initiated as a modified Ehrlich's test, according to Weil and Stieffel (placing the ligated forearm in cold water), but instead of local, general hemolysis occurred. The test of March 16, 1953 was performed after treatment with penicillin, and the test of May 14, 1953, was performed after treatment with cortisone.

The spectrographic determinations for hemoglobin were performed by Dr. K. Weber.

The patient's blood group was O, Rh positive.

Bleeding time was determined at 3 minutes, coagulation time (Bürker) 8 minutes, 10 seconds; retraction of blood clot occurred in one hour. The thrombocyte count (Fonio) was 250,800; reticulocytes were 25 per 1000 cells. Prothrombin time (Quick) was 100% of normal. The Rumpel-Leede test produced no cutaneous petechiae. The erythrocytes resisted hemolysis in saline dilutions from 0.52% to 0.58%. Bone marrow studies revealed an increased number of normoblasts. The Price-Jones curve showed erythrocyte diameters varying from 5.5 to 9.5 microns with the peak at 8 microns. Blood smear studies failed to reveal malaria parasites.

The Wassermann (WaR), Meinicke (MKR), Kahn (KR) and Citochol (CT) reactions for syphilis were all strongly positive. The WaR titer was 1:256. The treponemal immobilizing tests (Nelson Mayer) was positive*.

Radiologic examination of lungs, heart and abdomen revealed no recognizable lesions. Electrocardiographs were interpreted as normal.

*Rosenbach test.*³ The patient held her hands in cold water (about 10 C.) for 15 minutes then went to bed. About 30 minutes later she suffered shaking chills. At this time her temperature was 38 C. She complained of headache, nausea, abdominal cramps and lumbar pain. Neither urticaria or signs of Raynaud's disease developed. Table 1 shows the extent of hemolysis and hemoglobinuria. In addition to the hemoglobin, hyaline and granular casts appeared in the urine.

Mackenzie's rough test was negative. The blood was chilled for 10 and 30 minutes and kept at 37 C. for 15 minutes and 30 minutes, respectively.

*Donath-Landsteiner test*⁶⁻⁸ was carried out with 0.2 ml. of patient's serum and normal serum, 0.1 ml. physiologic saline solution and guinea-pig complement (serum was inactivated for 30 minutes at 56 C.).

To the serum was added 0.1 ml. of a 10% suspension of patient's erythrocytes or as control normal erythrocytes of group O. The erythrocytes were washed 3 times in physiologic saline solution at 37 C. The mixture was chilled on ice for 30 minutes then incubated at 37 C. for one hour. The reaction was positive at a serum and complement dilution of 1:128 (table 2).

* The TIT was performed by Dr. I. Orhel.

TABLE 2.—Donath-Landsteiner Test

| | |
|---------------------|--------------------|
| PS + PhS + PE = ++ | PIS + PhS + PE = 0 |
| PS + PhS + NE = +++ | PIS + PhS + NE = 0 |
| NS + PhS + PE = 0 | PIS + C + PE = ++ |
| NS + PhS + NE = 0 | PIS + C + NE = +++ |
| | INS + C + PE = 0 |
| | INS + C + NE = 0 |

PS = Patient's serum, NS = normal serum, PhS = physiologic solution, C = guinea-pig complement, PIS = patient's inactivated serum, INS = inactivated normal serum, PE = patient's erythrocytes, NE = normal erythrocytes. Positive hemolysis = +, ++ or +++.

TESTS

| Material tested | | Results |
|-----------------|--|--|
| Gastric juice | HCl Titration | Free acid 46 Total acid 52 |
| Stool | Benzidine reaction | negative |
| Stool | Stercobilin | negative |
| Blood and serum | Non-protein-nitrogen | 35 mg. % |
| | Urea nitrogen | 30 mg. % |
| | Bilirubin | Direct 0.8, indirect 0.48 mg. % |
| | Weltman reaction | 1-9 |
| | Thymol turbidity | 8 |
| | Thymol flocculation | 1 |
| | Cephalin cholesterol | negative |
| | Cholesterol | 220 mg. % |
| | Alk. Phosphatase (Bodansky) | 3.3 units |
| | Total proteins | 6.5 Gm. % |
| | Albumin | 4.32 Gm. % |
| | Globulin | 2.18 Gm. % |
| | | Electrophoresis Alb. 61%; Globulins: α_1 4.4%; α_2 5.46%; β 10.12%; γ 19.9% |
| Spinal fluid | Blood sugar | 98 mg. % |
| | Cells | 6/3 |
| | Pandy | Trace |
| | Nonne-Apelt | Trace |
| | Serologic test for syphilis (Wassermann, Kahn, Meinicke, Citochol) | negative |
| | Gold curve | Normal |

The blind test (without chilling) was negative. *The modified Donath-Landsteiner test** was likewise positive.

*Ham-test.*¹⁰ One ml. of a 5% suspension of erythrocytes was washed 3 times with physiologic saline solution, centrifuged and resuspended in a mixture of 0.95 cc. of serum + 0.05 ml., $\frac{1}{3}$ normal HCl. Controls were made with normal serum and erythrocytes. The mixtures were incubated at 37 C. for one hour. Hemolysis was absent.

* This is carried out with 5 drops of serum + 1 drop of 20% erythrocyte suspension placed on ice for 30 minutes. At 0 C. the erythrocytes are packed then washed twice with cold saline solution. After this 5 drops of physiologic saline solution and $\frac{1}{2}$ drop of active guinea-pig's serum are added and the whole incubated at 37 C. Kumagai and Inoue⁹ recommend washing of erythrocytes after cooling and addition of complement to remove anti-complemental substances.

Auto-agglutination occurred with both washed and unwashed erythrocytes at +4 C. At 37 C. there was no agglutination. Cold agglutinins were positive up to a 1:32 dilution.

Coombs test: The direct was positive. The indirect was negative.*

At present, signs or symptoms of syphilis are absent in the patient's parents, husband and daughter. The patient's father's serum when first tested gave a positive reaction in both the Wassermann and Kahn reactions. In later tests the Wassermann reaction was thought to be autotrophic and the other serologic tests were negative. At present even the Wassermann reaction is negative. The TPI was interpreted as a toxic reaction, when last performed it was \pm . Both patient's mother and husband are serologically negative for syphilis as is their TPI. The daughter's serologic tests are also consistently negative; however, the first TPI was apparently a toxic reaction, since repeat TPI tests are now negative.

Therapy

In January of 1953 the patient was given 15,000,000 units of penicillin. In April of the same year she received a total of 2.5 Gm. of cortisone. In addition she was given ACTH,¹¹ salvarsan and bismuth which had to be discontinued because of a severe reaction. In 1954, 15,000,000 units of penicillin were injected. Despite treatment there is no appreciable change in the patient's condition. Hemolytic attacks continue and repeated Donath-Landsteiner tests are positive. All serologic tests for syphilis (repeated 10 times) are strongly positive and the TPI test (3 times) is positive to a titer of 1:80.

DISCUSSION

This case corresponds in all details to classic descriptions of PCH. In most cases, syphilis^{3, 6, 12} is believed to be the cause. There exist, however, other opinions, and Soullier¹³ believes malaria is an important element in the etiology of PCH. Habersohn¹⁴ believes PCH may result as an abnormal reaction of the blood in syphilis, malaria, and other infectious diseases. Bernard¹⁵ does not believe the evidence supporting a malarial etiology is convincing.

Despite the history of malaria in our patient the weight of the evidence presented indicated that syphilis must be given prior consideration as the cause of the PCH.

Burmeister¹² has written that since hemolysins may be bound to cholesterol and lecithin as well as to extracts of syphilitic liver, the cold hemolysins, in this form, may give rise to a positive Wassermann reaction even in the absence of syphilitic reagin. He suggests the possibility that, in some cases of PCH the positive serologic tests for syphilis may be nonspecific and represent a more sensitive test than the Donath and Landsteiner reaction.⁶ Donath and Landsteiner believe that syphilitic reagin and cold hemolysins are unrelated. Becker³ has stated that the two can be separated. The Wassermann reaction may be positive in non-syphilitic diseases¹⁶ and methods have been devised to detect these false-positives (Kahn,¹⁷ Neurath¹⁸). Experience with the treponemal immobilization test indicates that it is specific for syphilis¹⁹⁻²² although it is possible to separate the syphilitic reagins from the immobilisins.^{23, 24} In view of the consistently positive serologic reactions for syphilis, particularly the treponemal immobilization test, it would seem reasonable to consider the etiology of the present case to be syphilis.

* The Coombs' tests were performed by Dr. A. Polak.

SUMMARY

A case of paroxysmal cold hemoglobinuria is described without a familial or personal history of syphilis. The patient, a 34 year old woman, shows no clinical evidence of syphilis. Numerous serologic tests for syphilis including treponemal immobilization are consistently positive. Despite therapy with penicillin and cortisone the hemolytic disease remains unchanged.

SUMMARIO IN INTERLINGUA

Es describe un caso de cryohemoglobinuria sin historia familial o personal de syphilis. Le patiente, un femina de 34 annos de etate, exhibi nulle signos clinic de syphilis. Numerose tests serologic pro syphilis, incluse immobilisation de treponema, es uniformemente positive. In despecto de tractamento con penicillina e cortisona, le morbo hemolytic persiste sin alteration.

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