

## From Ludwik Fleck's leukergy to the present-day rheology of leukocytes in heart and vascular diseases

Od leukergii Ludwika Flecka do współczesnej wiedzy na temat reologii leukocytów w chorobach serca i naczyń krwionośnych

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Zjawisko leukergii po raz pierwszy zostało opisane przez Ludwika Flecka, urodzonego we Lwowie w 1896 r. polskiego mikrobiologa i filozofa nauki, żydowskiego pochodzenia. Fleck był autorem ok. 170 prac oryginalnych i przeglądowych z zakresu mikrobiologii, serologii i immunologii.

Obecnie najczęściej wspomina się dorobek Flecka w zakresie filozofii i metodologii nauki, za sprawą wydanej w 1935 r. książki pt. *Powstanie i rozwój faktu naukowego*, pracy uznawanej za jedno z ważniejszych dzieł w tej dziedzinie wiedzy w XX wieku.

Fleck interesował się zmianami funkcji i morfologii leukocytów przez wiele lat swojej aktywności naukowej. W 1931 r. wspólnie z Altenbergiem ogłosił pracę pt. *Rozmieszczenie leukocytów we krwi w świetle rachunku prawdopodobieństwa*. W latach 1942–1952 wraz z Murczyńską opisał zmiany właściwości leukocytów występujące głównie podczas odczynu zapalnego w organizmie i wywołujące aglomerację tych krwinek w grupy jednorodnie cytologicznie (z powodu wzrostu ich lepkości), przyspieszenie przemiany oddechowej, ich ruchliwości i aktywności fagocytarnej. Prace Flecka na ten temat ukazały się zarówno w Polsce, jak i za granicą. Znaczenie odkrycia leukergii zostało uznane przez naukowców zagranicznych, a informacje na ten temat pojawiły się w najbardziej renomowanych czasopismach naukowych, takich jak *Journal of the American Medical Association* (1947) i *Lancet* (1947).

Celem pracy jest analiza współczesnej percepcji znaczenia odkrytego przez Ludwika Flecka zjawiska leukergii, szczególnie w odniesieniu do chorób serca i naczyń krwionośnych.

Stwierdzono, że pojęcie leukergii pojawiło się w bazie Medline w latach 1966–2006 w 35 artykułach, natomiast w bazie Pubmed w latach 1950–2006 w 125 artykułach, w tym w 12 bezpośrednio dotyczących chorób serca i naczyń krwionośnych. Znaczna część tych artykułów została opublikowana w ostatnich 15 latach w renomowanych naukowych czasopismach międzynarodowych. Sugeruje to potrzebę poszukiwania prostych i tanich testów oceny funkcji leukocytów, do których z pewnością należy test leukergii.

Jak wiadomo, funkcja leukocytów jest zaburzona w różnych chorobach serca i naczyń, w tym w niedokrwieniu mięśnia sercowego. Jest ona przedmiotem coraz głębszego zainteresowania i badań opartych na zaawansowanych technikach naukowych. Analizując wiele z tych badań, zwrócono uwagę na różnorodność nazewnictwa stosowanego dla określenia zjawiska zlepiania się leukocytów (leukergii). Obejmuje ono takie terminy, jak agregacja leukocytów, adhezja leukocytów czy reologia leukocytów. Utrudnia to znacznie śledzenie współczesnej percepcji znaczenia zjawiska leukergii w nauce. Jednak obserwowany wzrost zainteresowania reologią leukocytów w chorobach serca i naczyń krwionośnych, choć nie musi oznaczać renesansu terminu leukergia, z pewnością stanowi kontynuację badań polskiego uczonego Ludwika Flecka.

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## Life and work of Ludwik Fleck

The leukey phenomenon was first observed by Ludwik Fleck, a Polish scientist of Jewish origin, who was born in Lwów on July 11, 1896. He gained, as a student in the years 1920-1921, his first experience in microbiology as an assistant of Prof. Rudolf Weigl – a famous typhus researcher and inventor of the anti-typhus vaccine – at the Lwów University of Jan Kazimierz (UJK) [1]. After he received a medical degree from Lwów University in 1922, he specialized in bacteriology in Vienna. Then he became the head of the bacteriological and chemical laboratories of the State Hospital in Lwów. His expertise primarily related to the fields of microbiology and immunology. In 1935, Fleck published in Switzerland a book entitled *Entstehung und Entwicklung einer wissenschaftlichen Tatsache: Einführung in die Lehre vom Denkstil und Denkkollektiv* (Genesis and Development of a Scientific Fact: Introduction to the Study of Thought Style and the Thought Collective) in German. Only 200 books of 640 copies were sold. The book was discovered by Thomas Kuhn, philosopher and historian of science, and introduced to his *Structure of Scientific Revolutions* (University Chicago Press, 1962).

During the Second World War he headed the Bacteriology Laboratory at the Jewish Hospital in Lwów's Ghetto. In 1943 Fleck was arrested and imprisoned in the concentration camps, at first in Auschwitz, and later in June 1944 he was moved to Buchenwald [1]. At that time he continued his studies on typhus, trying to isolate the antigenic substance from the urine of typhoid patients which could be used as a vaccine for others. He described these early tests in the following words: *The search for specific antigenic substances in the urine of typhus patients was initiated in Lwów, 1942, under German occupation. The original plan was to elaborate a test giving earlier diagnosis than the Weil-Felix reaction... In addition to elaborating a diagnostic test, it was also thought to utilize the urine of typhus patients as a source of specific antigens for the preparation of a preventive vaccine, very urgently needed at this time... The author, his collaborators, and 32 volunteers were vaccinated... Later, 500 people in a concentration camp at Lwów were vaccinated... A large number of the vaccinated in the camp did not contract typhus although they were exposed to typhus infections* [2].

After the Second World War, Fleck settled in Lublin (since Lwów was 'ethnically cleansed' by the Soviet occupiers), where he headed the Institute of Microbiology of the School of Medicine at the Maria Skłodowska-Curie University, previously directed by the very famous Polish immunologist Prof. Ludwik Hirsztfeld

(also 'ethnically cleansed' from Lwów by the Soviet occupiers). In 1952 he moved to Warsaw, where he became the Director of the Department of Microbiology and Immunology at the Mother and Child State Institute, and, finally, in 1957 he emigrated to Israel. He died there in 1961, at the age of 64, of a second heart attack [1].

Although presently Ludwik Fleck is predominantly recognized as a philosopher of medicine and science, he was a very productive researcher in the fields of microbiology, serology and immunology. He published, as an author and co-author, about 170 original and review papers. His main achievements included discovery of the exanthin reaction, the detection of typhus antigenic substances in urine, the first description of the phenomenon of leukey, and many observations describing the behaviour of leucocytes in infectious and stress situations. The aim of this study is to analyze the perception of Fleck's major medical discoveries in present-day medical science, with special attention to heart and vascular diseases.

In the years 1923-1924 Ludwik Fleck conducted research on proteino-therapy in anafilaxy [3]. In 1930 he described the original skin test for early detection of typhus [4]. One year later he discovered some new *Proteus* strains of the X type in association with typhus, and introduced a new method of using *Proteus* extracts in agglutinin tests [5, 6]. In 1938 he demonstrated the influence of colloids on serological reactions and described a new method of distinguishing a real reaction from a pseudoreaction [7]. He described the lack or decrease of the fourth subunit of the component in luetic sera and studied the dependence of haematological pictures on rising agglutinin. He also wrote several papers on dermatology, focusing on lupus erythematosus, pseudophiloma and pemphigus [8-10].

In 1941-1942 Fleck discovered that typhus could be diagnosed before severe clinical symptoms were visible [11]. As early as the third day after the infection there would appear an antigen in the urine which could be discovered by means of Fleck's method. This was a valuable discovery, both theoretically and clinically, and it soon became well known in the Lwów medical milieu. Because of this, typhus (spotted fever) was anecdotally called 'Fleck fever', the name it already had in German ('Flecktyphus', *Fleck* meaning *spot* in German). He also developed a new method of typhus vaccine production, based on the presence of antigen in urine [2, 11]. He investigated the phenomenon of excretion of typhus antigens with urine and developed a method utilizing antigens for production of the vaccine against this disease [2, 11].

One of his late microbiological reports concerning the latex agglutination test with *Brucella* antigen was

published in Nature in 1962 [12]. In this paper he described the test, which permitted the detection of minute amounts of Brucella antigen.

However, the description of the leukergy phenomenon became his main achievement in the field of immunology. The name leukergy was derived from two words: leukocyte and *ergein* (action in Greek), which meant activation of the leukocyte system. Fleck described changes in the leukocyte properties due to the inflammation process in living organisms. These changes included the aggregation of leukocytes in homological (or homologous) cell groups (an effect of increasing their viscosity), an increase of oxidation turnover, their migration and phagocytic activity. Leukergy was first observed in 1942, and then described as *an inflammatory phenomenon which can be produced in animals by the intravenous administration of killed bacteria. Tests we have done suggest that there was no link between leukergy and leukocytosis, body temperature and erythrocyte sedimentation rate (ESR). It appears several hours after the injection of bacteria, subsequent to the rise of temperature, but before leukocytosis. It lasts about five days, that is, much longer than the length of fever, usually longer than leukocytosis and increased ESR. Leukergy is a very sensitive symptom. (...) While ESR is a strictly humoral phenomenon, i.e. the red cells of a sick patient suspended in serum from a healthy person settle normally, and the red cells from a healthy person suspended in a patient's serum settle fast, leukergy is something different. In leukergy, white cells centrifuged from the inflammatory serum, washed with serum from a healthy person and settled in such serum, remain leukergic* [13, 14].

Fleck devoted many articles to the investigation of leukergy and the function of leukocytes in health and disease [15-18]. He discovered, among other things, antibodies against leukocytes in the blood of mammals and assessed their importance in the formation of characteristic haematological pictures. Moreover, he studied the leukocyte oxygen processes during phagocytosis.

Although many handbooks of bacteriology and immunology from around the middle of the 20<sup>th</sup> century contained Flecks' papers, it seemed interesting to check to what degree his scholarly contribution has been reflected in the newest literature of the subject. For this purpose the Medline base was used, with the following headwords: leukergia (35 records), Ludwig Fleck (35 records), and exanthin reaction (0 records). The search period involved the years from 1966 to 2006. There are also over 400 entries for 'leukergy', four for exanthin and 149 000 for 'Ludwig Fleck' in Google.

The other medical literature database, Pubmed, for the headword 'leukergy' in the period 1950-2006, revealed 125 records. These records were divided chronologically in ten-year periods, which revealed 38 records in the years 1950-1959, 32 records in the years 1960-1969, 28 records in the years 1970-1979, 7 records in the years 1980-1989, 16 records in the years 1990-1999, and 4 records in the years 2000-2006. All of these records were categorized into 13 medical areas, as follows: inflammation (30), neurology (15), immunology (14), vascular and coronary diseases (12), paediatrics (7), orthopaedics and surgery (6), psychiatry (4), microbiology (3), septic shock (2), gynaecology (2), ophthalmology (2), trauma (1), and others (27), including single and non-classified areas, like smoking (1), chronic alcoholism (1), and general reviews (25).

The review of the aforementioned publications revealed that the leukergy test was used in the many different fields of medicine, including infectious disease, circulatory and coronary diseases, inflammatory diseases, smoking-related disorders, immunological disorders, occupational medicine, monitoring of neuroleptic drug use and ophthalmology. Different experimental and medical indications of the leukergy test are discussed below.

### Leukergy in vascular and heart diseases

The role of leukocytes in myocardial ischaemia and reperfusion has been extensively studied and described elsewhere [19, 20].

There is not so much direct evidence for use of the leukergy test in the field of circulatory and coronary diseases. The leukergy test was used to evaluate the microvascular response in patients with cardiogenic shock [21], and as a test for the evolution of cerebral ischaemic injury [22] and previous cerebral ischaemic event [23].

It was also shown to be a good test for neutrophil activity in patients with myocardial ischaemia, stable angina pectoris and treatment with certain drugs [24, 25], for leukocyte rheological properties in patients with diffuse atherosclerosis and for leukocyte aggregation evaluation in ischaemic heart disease [26]. Berliner et al. concluded that the leukergy test correlates better with the clinical picture than does the NAA test (the neutrophil aggregation activity test) [27]. Moreover, it is believed that the NAA test indirectly examines the aggregation potential of the plasma more than the aggregation state of the patient's leukocytes. The leukergy test was also used for the assessment of leukocyte function in patients with myocardial infarction infected by *Helicobacter pylori* [28].

It was also proposed by using the simple slide test of leukocyte adhesiveness and aggregation (leukergy test) that increased state of adhesiveness and aggregation of leukocytes may contribute to the development of ischaemic vascular conditions in diabetic patients [29].

It should also be noted that many studies concerning changes in adhesiveness and aggregation of leukocytes did not mention the leukergy test, partly because in the last three decades there have been many different modifications introduced to the method and partly because some authors may even be unaware of the historical aspect of the test. The other point is that the leukergy test, although rather simple and inexpensive, might be treated as inadequate for present-day laboratory needs. Nowadays, there are certainly better and more detailed tests to study rheological properties of leukocytes, for example membrane fluidity and cytosolic  $Ca^{2+}$  concentration. It was shown that disturbances in these parameters occur in patients with myocardial infarction and may contribute to the prognosis of the disease [30]. It was shown that adhesion of polymorphonuclears (PMN) is mediated by adhesion molecules like ICAM-1, L-selectin and E-selectin, and that expression of these molecules influences the myocardial infarct size [19].

The Pubmed search for 'neutrophil aggregation and heart disease' revealed 128 records, and for 'neutrophil aggregation and heart' 103 records, although these words occur in many different combinations, like 'leukocytes (PMN) aggregation', 'leukocyte rheological properties', 'erythrocyte and leukocyte aggregation', etc.

## Conclusion

It seems that Fleck's observation of leukocyte activation and aggregation during inflammation is still relevant in science, even if it is not named leukergy. There are various modifications of the original Fleck method, such as the leukocyte aggregation/adhesion test (LAAT), and different terms used to describe the same phenomenon, like aggregation of leukocytes or leukocyte rheological properties. This shows the difficulties of tracking and analyzing Fleck's direct influences on present-day medical science.

However, the analysis of the available literature revealed growing interest among medical researchers in the role of leukocytes' rheological properties in inflammation, with special recognition of its significance in heart diseases. Although the leukergy test is said to be a non-specific method, it is also sensitive, simple, and, what is now becoming increasingly important, inexpensive. Moreover, this analysis showed that the majority of articles in prestigious and peer-review journals concerning the leukergy test have been

published during the last 15 years. On the other hand, a lack of clear and standardized diagnostic indications for the test's use in clinical conditions makes it still an experimental rather than a clinical method. This means that there is still ample room for research in this area for Ludwik Fleck's followers.

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