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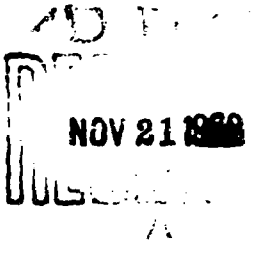

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

THE SERODIAGNOSIS OF SYPHILIS BY IMMUNOFLUORESCENCE.
(in regard to 307 examined sera)

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In 1942 Coons discovered the immunofluorescence technique: the proteins and, in particular, the serum globulins, can be combined with fluorescent substances, as fluorescein isothiocyanate. The thus "marked" globulins retain their immunological properties and seem fluorescent when examined under ultraviolet light. Thus, they can be easily detected when, because of the antibody function, they fix themselves on figurative antigens, bacteria, parasites or viruses.

Two procedures are usable:

--the direct method, in which the combined proteins are the globulin-antibodies of a serum prepared against a well defined antigen germ: the antigen-antibody union thus allows the detection of the antigen on which the antibody is fixed. But this method requires the marking of each serum to be examined, a long and delicate job because the serum must be very pure. Also, was it replaced by:

--the indirect method, called "sandwich", which consists in

revealing the formation of an antigen-antibody complex by means of an antiglobulin coupled with isothiocyanate and corresponding to the species of animal to be examined. This antiglobulin fixes itself on the antibody, which is itself already fixed by the antigen (that is to say the germ) and makes it fluorescent.

Thus, in 1957, Deacon Falcone and Harris were the first to apply this technique to the serodiagnosis of syphilis.

The serum of a syphilitic subject contains antitreponema antibodies which are globulins. If one fixes a treponeme (for example treponemes of Nichol's strain, identical to those used in Nelson's test and obtained by passages through rabbit testicles) suspension on a slide, these globulin-antibodies will fix themselves on the spirochete, but also act as antigen for an antiglobulin-human serum which also fixes itself on the treponeme antigen-antibody complex. Since the antiglobulin-human serum is combined with a fluorescein derivative, the treponeme becomes fluorescent under ultraviolet examination. On the other hand, in the absence of antibody, that is to say in the study of a nonsyphilitic serum, the antiglobulin is not fixed and the antigen remains nonfluorescent.

Study material and techniques

To date, we have studied 207 sera which have all undergone classical serological examinations with cardiolipid and treponeme (Kolmer, Reiter and Kline) antigens; with most we were also able to perform a Nelson's test.

These sera were divided thusly:

--50 primary syphilis sera;
--23 secondary syphilis sera;
--3 tertiary syphilis sera (2 cardiovascular, 1 neurological);
--80 sera of so called "serological" syphilis;
--lastly, 51 sera of subjects free from syphilis, having a classical negative or positive or dissociated serology, but a negative Nelson's test.

The immunofluorescence test (F.T.A.) was applied:

Note: F.T.A. stands for Fluorescent Treponemal Antibody Test.

--either to sera obtained by decantation of coagulated blood, for the 118 examinations from the H.M.I. (Military Hospital of Instruction) at Val-de-Grace;

--either to dry blood for 89 examinations sent from various military hospitals (Toulouse, Lyon, Versailles, Rennes, Grenoble, Toulon). This method of sampling, recently detailed by Vaisman and Mrs. Paris-Hamelin[1] in 1963, is particularly interesting because it allows blood to be mailed to a distant laboratory. The blood is taken by sticking the finger pulp and absorbing directly on a round of Cranson No. 435 paper having a diameter of 15 millimeters; the rounds must be completely impregnated and left to dry for two hours in free air; in the laboratory they are reconstituted in a pH 7.2 buffer solution for two hours. The quantity of buffer is sufficient to give the equivalent of a 1/100dilution of fresh plasma.

The immunofluorescence technique we used is strictly that of Vaisman and Mrs. Paris-Hamelin at the Institut Alfred-Fourner de Paris (Alfred-Fournier Institute of Paris).[2] References for all the technical details can be found in the bibliography.

Very schematically, the method consists in placing a drop of treponeme suspension in a 10 millimeter circle cut on a slide. When this has been fixed by acetone, it is covered with a previously diluted drop of the serum to be examined. After fixation for 30 minutes at 37° C in a humid atmosphere and a careful washing, a drop of antiglobulin-human serum diluted to the proper titer is placed on top. Following another fixation in a humid chamber and rinsing, the preparation is mounted under a cover slip in buffered glycerinated wa r. The slides used must be as thin as possible to absorbe the least possible luminous intensity and the examination must be made under ultraviolet light and dark field.

The most delicate reaction times are certainly the various fixation times, because the preparation must not dry.

Each serum was qulitatively studied at the 1/100 dilution: the limit dilution to be used according to various authors, above which some "false reactions" can appear. The reaction can be quantitative.

Before use, the sera are decomplemented and even left for an hour at 56° C to take into account the remarks made by Thivolet and Cherby-Grospiron.[3]

This technique gave us complete satisfaction in that which concerned the sensitivity as well as the specificity of the test even though it was a question of a reaction which was rather delicate and difficult to use on a large number of samples at the same time.

Often there is the complaint that with this method, the results cannot be reproduced and that the marked antiglobulin is not standardized. This is a fact because, even coming from the same laboratory, the marked antiglobulins are used at different dilutions according to the lot. In reality each lot of antiglobulin can be titrated in relation to dilutions of a positive syphilitic control sera of known titer, a serum remaining stable by preserving it at -20° C.

Taking these precautions into account, what advantage does F.T.A. have in relation to classical serology and Nelson's test, considered here at various stages of syphilitic infection and taken with reservation because of the modest number of examinations made to date in the laboratory?

Results

For the diagnosis of primary syphilis, F.T.A. seems to have a great advantage. Actually, the fluorescent antibodies appear earlier than the other antibodies.

In 50 cases of primary syphilis studied here, for which F.T.A. was always strongly positive (++++ or +++), classical serology found:

--34% positive;
--35% dissociated;
--31% negative.

The ultramicroscope examination was positive in only 23% of the cases. Of these 50 sera, 16 were given Nelson's test: it was only positive three times, twice at 50 to 60% specific immobilization titers. That is to say that for one case in three of primary syphilis, the only proof of the existance of the affliction is F.T.A.

If account is taken of the fact that a dissociated classical serology (that is to say positive, either in complement fixation or floculation) only brings doubt not certitude, it is in 66% of the cases, or two out of three times that F.T.A. has made the diagnosis. It is known that Nelson's test is only rarely positive in the primary stage.

Elsewhere, it seems that during treatment (penicillin alone or penicillin therapy followed by bismuth cure), the fluorescent antibodies disappear much more slowly than the reagins. We have found nine positive F.T.A. while the classical serology was completely negative. In four cases, the F.T.A. were found to be positive eight to nine months after classical serology became negative. These

responses could cause therapy to continue.

In the secondary stage, F.T.A. as well as classical serology and Nelson's test, is regularly positive.

The 23 secondary syphilis sera were all strongly positive with F.T.A., as were the current serology reactions (except for one serum giving dissociated responses).

Nelson's test performed on 15 sera was positive 11 times with 100% immobilization. The disagreement with F.T.A. thus concerned four sera, in favor of the latter. It was a question of so called "primo-secondary" syphilis, where the choice of method seems very important: actually, in these four cases, treated precociously, classical serology was negative and Nelson's test never became positive.

During treatment of the so called secondary forms, we noted five times that classical serology was negative or dissociated, while Nelson's test and F.T.A. both remained positive.

Thus, if from a diagnostic view point F.T.A. has no great value in the secondary stage, it is as advantageous as Nelson's test in the surveillance of treatment and can even be superior to it, when the latter has not had time to become positive.

In the case of the search for a syphilitic etiology of a syndrome capable of revealing the tertiary stage of the disease, it is difficult to define the advantages of F.T.A. because we only had three sera to examine and they were all positive ++++. For two of them classical serology was positive as well as Nelson's test; for the third examination made because of an anginous syndrome with strain, classical serology was completely negative, but Nelson's test was 100% positive.

These results are thus in agreement with the conception according to which, at these stages of the disease, F.T.A. and Nelson's test evolve in a similar manner.

It is the same for so called "serological" syphilis. Actually, in the examination of 80 positive F.T.A. sera revealing this rubric, we found the following classical serology results:

--66% positive;
--34% dissociated.

All the Nelson's tests performed, in particular the dissociated serological forms, or 45 tests, were positive.

Treatment followed by an often long time, gave negative classical serology in eight patients while Nelson's test and F.T.A. remained positive.

Attention is called to the case of a patient whose classical serology was first negative, followed Nelson's test one year later, and who still presently has a positive F.T.A. ++++.

As to the specificity of the immunofluorescence test, we have recorded no false positives in the 47 control serums which we studied. On the other hand we have noted the negative F.T.A. and Nelson's test for four sera which had false positive classical serology reactions: sera of patients having primitive atypical pneumonia, dermatosis, filariasis and an annular lesion of a gland of undefined etiology.

Conclusions

This new test for the serological diagnosis of syphilis should take its place next to Nelson's test; it is already held in great favor in the United States and in the U.S.S.R.; in France, Fribourg-Blanc, Vaisman and Mrs. Paris-Hamelin, as well as some other authors contribute to its spread.

It seems to have the same specificity, reliability and sensitivity as Nelson's test; it even has an advantage over the latter in that it can track down treponeme antigens, doubtlessly different from the immobilisins, which appear the most early of all the antibodies.

It can be applied to sera which are useless in the immobilization test as well as sera called "toxic", more and more frequently found and very often due to the absorption of antibiotics or tranquilizing medications. It is the same for sera coming to the laboratory in a nonsteril condition and for which Nelson's cannot be used.

Lastly, it represents a simplification technique. While requiring much care in carrying out, a certain minuteness, there is no need of a survival media for the treponemes which is always difficult to do and irregular in its value; the Nichol's strain is actually used killed and the antigen suspension keeps a long time in the refrigerator.

The principal complaint made of the method is certainly the lack of standardization in the antiglobulin serum, which presently requires long

titrations when the lot is changed. Already real progress has been made in correcting this. It is a method which can still be improved.

(Work of the serological laboratory at the military hospital of instruction at Val-de-Grace.)

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