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THE FTA IMMUNOFLUORESCENCE TEST FOR SYPHILIS

Bollettino delle scienze mediche (Bologna) Arturo Longhi
(Medical Sciences Bulletin [Bologna])
Vol. 136, pages 246-250 (1964)

[This paper was presented by the author on 9 April 1964 at the scientific conference held by the Bologna Medical Surgery Society (Società Medica Chirurgica di Bologna)].

The subject of my paper, dealing with blood tests for syphilis, was not selected by mere chance for this conference, dedicated by the Bologna Medical Surgery Society to the memory of Leonardo Martinotti.

Martinotti did, in fact, interest himself in serological research in connection with syphilis, particularly from the standpoint of treatment and he also improved upon a quantitative method of the well-known Wassermann reaction to obtain a more accurate meaning of specificity and sensitivity. Now, though, the serological problem of syphilis presents itself in quite a different guise from the time of Martinotti because of the new knowledge gained about the complex antigenic constitution of *treponema pallidum*, the ever more selective research on the antibodies evoked by the various antigenic fractions of *treponema* and the studies that have emphasized the various antibody-genetic possibilities associated with the composition of an individual's blood proteins. Therefore an attempt has been made to give greater attention to increased accuracy in carrying out blood tests for syphilis; new methods and new reactions have been experimented with (agglutination test, immuno-adherence test, complement deviation with antigens derived from protein extracts of the *treponema*, Nichols strain or Reiter strain, microfloculation with cardiolipin antigens, etc.) and the FTA [abbreviation not explained in text] immunofluorescence test and the Nelson-Mayer immobilization test (TIT [treponema immobilization test] or TPI [treponema pallidum immobilization]) have given added significance and meaning and more accurate information about the immuno-allergic situation associated with the disease and about prognostic and therapeutic problems.

The Nelson-Mayer test (treponema pallidum immobilization test) already has been presented widely in the literature, documented by a large number of case histories and a long bibliography and its good specificity and sensitivity are recognized by all, although there are a number of technical difficulties involved in preparing the material, taking readings and interpreting the results, particularly disadvantageous for its use as a routine test. The immunofluorescence test, more recently introduced (the first works of Deacon, Falcone, Harris appeared in 1957 but it was not until 1960-1961 that the reaction gained wider use) is still going through its stage of critical development but its great importance and its deep significance can already be appreciated. The principle on which the reaction is based, applicable to research on every kind of antibody, is very simple and consists in microscopic examination under ultraviolet light of an antigen-antibody complex rendered fluorescent by fixing the antibody with a fluorescein derivative. There are two possible methods of carrying out the test, namely, either fix the fluorescent substance to the antibody by treating the serum being examined directly (direct method) or use an antiglobulin (rabbit, goat or other anti-human serum) tagged by fluorescence to reveal the antibodies (human globulins) fixed to the treponema (indirect method). The latter is the most often used method and is the one used by us.

I will not go in any detail here into the questions of a technical nature of interest to laboratory technicians but it is necessary to emphasize the fact that even if it is relatively easy to carry out the test it is still very delicate and requires the use of equipment of the type found only in big laboratories, particularly with respect to the microscope apparatus, to prepare the reagents and to take the readings which should, in so far as possible, always be taken by the same person (in clinical treatment the reading is carried out for each reaction by two or three persons using two samples of the same serum being examined). Though we recognize that this test offers a high degree of sensitivity, specificity and repeatability, we have not given up the conventional serological reactions so that together with the FTA test we also carry out the other well-known reactions of complement deviation with organ antigen and with cardiolipin and the flocculation test (Weinicke, Kahn, VDRL [abbreviation not explained in text -- possibly Venereal Disease Research Laboratory], microfloculation with cardiolipin antigens). We also carried out the Nelson-Mayer test in cooperation with the Modena Dermatological Clinic on many sera, particularly

in the beginning. Now we have a collection of many well documented case histories enabling us to draw conclusions about the importance and the real and practical significance of the immunofluorescence test in blood tests for syphilis and in the study of the state of the disease from the standpoint of immunization.

For a more specific evaluation, I am reporting in the following on the behavior of the test during the various stages of clinical development of treponemic infection.

In primary pre-serological syphilis (that is, with chancre and the usual blood tests still showing negative) the FTA test gives a positive result at a very early date and in some cases even only three or four days after appearance of the chancre. This reaction always precedes the reactions occurring in the other, conventional tests, including the flocculation reactions which are the first among the conventional tests to show positive. The Nelson-Mayer test shows positive with a longer delay. From the practical standpoint this early positive reaction to the FTA test is important for those cases in which one cannot obtain microscopic evidence of treponema in the primary lesion (for example, because it has been treated locally with antibiotics, etc.).

In primary serological syphilis, that is, in those forms of initial syphilis with chancre dating back some weeks or already receding but untreated and with positive test results using conventional blood tests, the FTA test always shows positive while the TIT fails to react or if it does it shows low percentages of immobilization (20 to 30 per cent). In those forms that have been subjected to treatment and still in the primary serological period, the conventional tests can still show negative even after several months of treatment but not the immunofluorescence test nor the immobilization test, either, which turned positive at a somewhat later time. If further cycles of treatment are given after a first cycle one can attain a slow weakening of the reactivity of the two tests until they both become entirely negative which could also indicate that the patient has been cured (though naturally the patient will still continue to be followed up and checked up on with the passage of time.

In secondary syphilis with skin or mucus membrane symptoms all the serological reactions show positive, including the FTA and the TIT. Treatment undertaken during this stage of the disease, if carried on properly and continued over a long period can lead to completely negative reactions (which, if they persist so, indicate that the patient has been cured clinically).

In secondary syphilis without any apparent symptoms, that is, in syphilis contracted no longer than four years beforehand, if the patient has not taken any treatments or has treated himself improperly the FTA and TIT tests will always show positive; if the patient has undergone treatment then depending on the intensity, regularity and duration of such treatment the FTA test will gradually taper off until it becomes completely negative after three to four years. The treatment clearly influences the behavior of the test results; one can even specify that the test results will become negative earlier to the extent that treatment is begun at an earlier date, while the later treatment begins and the more irregularly treatment is carried on then the greater will be the percentage of positiveness of the test.

In tertiary syphilis with central nervous system involvement (tabes, progressive paralysis) the FTA test is positive for both the blood and the spinal fluid in a large percentage of cases (94 per cent) as is also the Nelson-Mayer test, while the conventional blood tests may sometimes show negative (70 per cent positive). In other situations with lesser symptoms like aortitis, neurological-visceral syndrome and developing more slowly wherein even in autopsies one seldom observes proliferative foci with treponema the conventional serological reactions are often negative and the FTA test can be weakly positive or sometimes even negative (the Nelson test also behaves similarly).

In late syphilis, that is, in old syphilitics without any apparent symptoms the FTA test is highly important for curing the disease. Even in well-treated cases the FTA test can still show positive in even a considerably high percentage (55 per cent) (compared to the Nelson test 19 per cent and the conventional tests 3 per cent); in poorly treated cases the percentage of positive results increases to the point of becoming absolute in cases that have never been treated or in those patients having latent syphilis. If there is no doubt about the significance and the interpretation of the response to the test using immunofluorescence in cases of poorly-treated or untreated late syphilis and latent syphilis (we certainly must admit that in such cases the infection is still active and it is necessary to carry on specific treatment), in cases of properly treated latent syphilis if the FTA shows negative I think we must say that the disease has been cured, but when it is still active it will be well to proceed by still treating the patient and attentively monitoring the changes in serological response under treatment to decide whether one should still continue with specific treatment. If the immunofluorescence reaction following treatment shows a slow tendency towards

recession we try to persist in treatment until complete disappearance of the immunofluorescent antibody (if possible); if, instead, in the presence of absolutely no clinical record and no symptoms the test shows no change and remains positive even after prolonged, intense treatment, one can accept with some reservations the concept of a serological scar and even though not recognizing any absolute cure, one can admit that the latent infection is no longer such but instead has reached a state of commensalism wherein the absence of clinical symptoms is more important than the continued positive test reaction and where the balance that has been established can remain as it is even for the rest of the patient's life.

In congenital syphilis the immunofluorescence test has been found positive in 57 per cent of the cases (somewhat below the TIT test reaction in 70 per cent of cases), even in treated patients without any appreciable evidence of clinical symptoms. These cases, too, raise questions of a serious nature with respect to treatment and prognosis; the problem is this: should these patients be treated or not? Our experience teaches us that even after treatment the reaction still persists positive in many cases so it should be held that such a positive test reaction is not an expression of any disease following an active course but rather an expression of indelible serological scars which remain unchanged with treatment.

Non-syphilitic sera of patients affected by lupus erythematosus, leprosy, viral infections, irregularly reacting diseases, etc. that showed false reactions with positive blood tests responded to the FTA tests by always showing a negative result (the same as in the TIT test, too).

From all of these findings reported on the different stages of treponemic infection it appears evident that the FTA test is provided with optimum specificity and sensitivity in the different stages of the disease. Highly important is the earliness of the response in primary syphilis when the conventional blood tests have not yet begun to give any indication and when the Nelson-Mayer test is still negative. The immunofluorescence test represents an important diagnostic means in cases with doubtful medical histories, uncertain clinical symptoms and indefinite blood test results but above all this test finds specific application in evaluating the results obtained with specific treatment and in judging whether the patient has been cured clinically and immunobiologically. If one considers the fact that in a high percentage of situations this test practically duplicates the results of the Nelson-Mayer test while differing from it in

primary syphilis in giving an earlier positive test response and in congenital syphilis in giving a less reactive response and if one also bears in mind its greater simplicity from the technical standpoint in preparing the tests, one would conclude that it can be broadly applied in carrying out routine blood tests for syphilis with a broad margin of biological safety and with extensive practical possibilities.

Other members of the society, Prof. C. Manzini, P. Parisi, A. Guarino and Prof. E. Sega commented on the paper and were answered by the speaker.

The meeting was adjourned.