

Antinuclear Factor in Rapid and Slow Acetylator Patients Treated with Isoniazid

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It has been observed that tuberculous patients who had been treated with isoniazid exhibited positive antinuclear factor (ANF) tests more commonly than controls (Cannat and Seligmann, 1966). ANF can be considered a forerunner of the drug-induced systemic lupus erythematosus syndrome (Alarcón-Segovia, 1969).

Both isoniazid and hydrallazine have been demonstrated to be polymorphically acetylated in man (Evans, 1968).

Perry, Sakamoto, and Tan (1967) and Perry *et al* (1970) have shown that the development of antinuclear factor in patients who have received treatment for hypertension with hydrallazine is more frequent in slow than in rapid acetylators.

In view of the above observations one of us (D.A.P.E.) suggested a survey of tuberculosis patients under treatment for serum ANF, acetylator phenotype, and other possible relevant factors.

Materials and Methods

Patients. Volunteer patients were investigated either as inpatients at Aintree Hospital, Liverpool or shortly after discharge when they were continuing their treatment as outpatients under clinic supervision. (There were three exceptions, all ANF negative, in which there were intervals of 2, 3, and 5 months between cessation of isoniazid therapy and the drawing of the blood specimen for ANF determination.) All available volunteer patients available during the period of the study were investigated (ie, there was no selection).

Sulfamethazine Phenotyping Procedure was as described in series I of Evans (1969). Fasting patients swallowed 160 mg sulfamethazine (syn. sulphadimidine) per kg metabolically-active mass (ie, weight to the power of 0.7) as crushed tablets with a little water. Two hours after drug ingestion the patients were allowed tea (or coffee) and toast. Thereafter normal meals were

allowed. All urine excreted during 8 hours following drug ingestion was collected, and a portion stored at -20°C to await analysis. A sample of venous blood was taken at 8 hours following drug ingestion; the serum separated and stored at -20°C to await analysis.

For three patients the dosage and timing of the collection of the specimens were modified as detailed in series II of Evans (1969).

Estimation of Sulfamethazine Concentration in Urine and Serum. When the samples were thawed care was taken to ensure that the sulfamethazine and acetyl sulfamethazine were completely dissolved before analysis was performed. For this purpose urine samples were placed after thawing in a boiling water bath for 5 minutes, and thawed sera were placed in a 37°C incubator for 1 hour.

Free (F) and total (T) sulfamethazine concentrations were estimated in unknowns in duplicate and a range of standards at 2, 4, 6, 8, and $10\ \mu\text{g}$ sulfamethazine per ml, together with suitable blanks, were processed in duplicate simultaneously.

The percentage of sulfamethazine acetylated is given by the formula:

$$\frac{T-F}{T} \%$$

(see Evans and White [1964] for further details of the analytical technique).

Blood Samples for ANF Testing. Sera were stored at -20°C and transferred frozen to the Department of Pathology, Western Infirmary, Glasgow. Information concerning acetylator phenotype was not available to the researcher (J.M.V.) who carried out the ANF tests.

ANF Tests. These tests were carried out as described previously (Wren *et al*, 1967). In each batch of tests known positive and negative sera were included to act as controls.

Statistical Techniques were standard (Bailey, 1959).

Results

Acetylator Phenotyping. The results of all patients, except 4, are shown in the Figure.

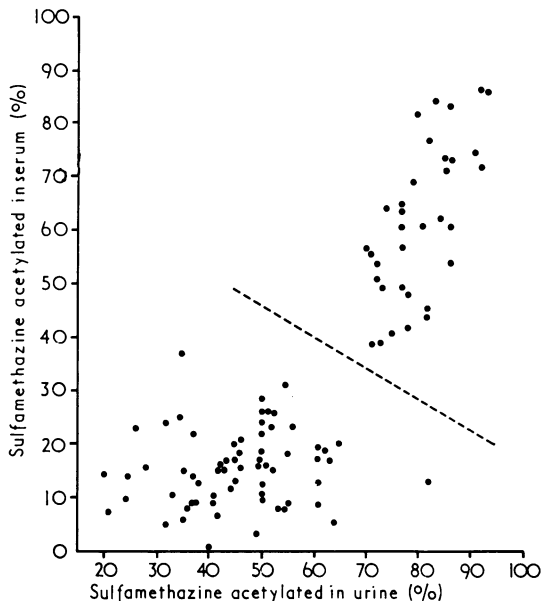


FIG. Acetylator phenotyping data on 99 patients. The dotted line separates slow and rapid acetylators. As explained in the text, the results of 4 patients are not shown on this scattergram.

The details concerning the 4 exceptions are as follows.

For one patient only the serum sample was available. The percentage of sulfamethazine in his serum was 17.05% and so this patient had been categorized a slow acetylator. Three patients were phenotyped by a modified technique (as series II of Evans, 1969) and they gave results indicating that two were slow acetylators and one a rapid acetylator.

Antinuclear Factor was found in the sera of 17 patients (16 male), whereas the sera of 86 patients (76 male) gave a negative test. As indicated in Table I there is no significant association between sex and ANF in the patients in this survey. ANF is usually found to occur more frequently in women than in men. The present series of patients, however, contained only a minority of women, and this fact may well account for the failure to demonstrate the usual sex incidence of ANF.

Six patients (all ANF negative) had been observed to be unreliable in taking their medications. One subject (ANF negative) had isoniazid-resistant tubercle bacilli and had been receiving isoniazid and

TABLE I

SEX AND THE PRESENCE OF ANTINUCLEAR FACTOR IN TUBERCULOSIS PATIENTS

Antinuclear Factor	Male	Female	Total
Positive	16	1	17
Negative	76	10	86
Total	92	11	103

Fisher-Irwin exact probability 0.42.

other drugs for 4 years, the quantities not being precisely known. He had received isoniazid during the hospitalization within the period of this study for 6 weeks, ceasing 5½ months before the blood for ANF determination was drawn. One subject (ANF positive) had received isoniazid for only one day before blood for ANF determination was drawn. These 8 subjects are excluded from the following results.

Full clinical details of satisfactory quality were available on 67 patients.

The disposition of ANF-positive results by acetylator phenotype is shown in Table II. There is no significant association.

TABLE II

ACETYLATOR PHENOTYPE AND THE PRESENCE OF ANTINUCLEAR FACTOR IN TUBERCULOSIS PATIENTS

Antinuclear Factor	Acetylator Phenotype		Total
	Rapid	Slow	
Positive	4	12	16
Negative	28	51	79
Total	32	63	95

$\chi^2 = 0.65$, $p > 0.10$.

In both rapid and slow acetylator phenotypes the ANF-positive patients were older than the ANF-negative patients (Table III). The mean age of 16 ANF-positive patients was 59.81 years (SE 2.27); and for 79 ANF-negative patients the mean age was 42.85 years (SE 1.74). The tests for the significance of the difference between the means gives $t = 4.23$ and $p < 0.001$.

The length of exposure to isoniazid therapy (total range 22 to 112 weeks) and total dosage of isoniazid (total range 44 to 236 g) consumed were not significantly different in ANF-positive as compared to ANF-negative patients when sub-divided by acetylator phenotype (Table III).

The drug most commonly used long-term with

TABLE III
DATA ON PATIENTS WITH TUBERCULOSIS

Acetylator Phenotype	Anti-nuclear Factor	Age (yr)		Weeks on Isoniazid		Weight Isoniazid Consumed (g)	
		No.	Mean ± SD	No.	Mean ± SD	No.	Mean ± SD
Rapid	Positive	4	59.25 ± 11.67	4	62.44 ± 28.53	4	131.10 ± 59.93
Rapid	Negative	28	45.61 ± 16.32	20	51.40 ± 17.46	20	105.57 ± 38.73
Slow	Positive	12	60.00 ± 8.64	12	45.08 ± 19.12	12	94.56 ± 40.01
Slow	Negative	51	41.33 ± 14.92	31	51.84 ± 24.31	31	108.76 ± 51.17
F			6.45		0.68		0.67
df			3, 91		3, 63		3, 63
p			< 0.001		> 0.20		> 0.20

isoniazid was para-aminosalicylic acid (PAS). In the group of ANF-positive patients three were on drugs other than PAS together with isoniazid—namely ethambutol, cycloserine, and pyrazinamide. In the group of ANF-negative patients 14 had other drugs regularly in addition to isoniazid and PAS, and the drugs concerned are listed in Table IV. Almost all patients were also treated with a short initial course of streptomycin.

TABLE IV
DRUGS OTHER THAN PAS TAKEN WITH ISONIAZID BY ANF-NEGATIVE PATIENTS

Short Courses of Streptomycin	(Most Patients)
Prothionamide	(1)
Pyrazinamide, prothionamide, and Librium	(1)
Ethionamide	(1)
Cycloserine	(4)
Thiacetazone and cycloserine	(1)
Tolbutamide	(1)
Pyridoxine and Intal	(1)
Thiacetazone	(1)
Pyridoxine and Ferrogradumet	(1)
Thiacetazone, pyrazinamide, and cycloserine	(1)
Librium	(1)

Disorders other than tuberculosis were of frequent occurrence in the patients surveyed, both ANF positive and ANF negative. None of the associated disorders noted in the ANF-positive patients were conditions known of themselves to be associated with a raised incidence of ANF (see Table V).

Discussion

The incidence of ANF in the tuberculosis patients in this survey was approximately 17% and this is considerably higher than the incidence commonly found in random population surveys. For example, in the paper of Wren *et al* (1967) amongst 66

TABLE V
DISORDERS OTHER THAN TUBERCULOSIS RECORDED AS PREVIOUS ILLNESSES IN 16 ANF-POSITIVE PATIENTS

Condition*	Times Recorded (n)
Pleurisy	1
Peptic ulcer	1
Duodenal ulcer with operation	3
Myocardial infarction	1
Lichen simplex	1
Herpes zoster	2
Multiple sclerosis	1
Pneumonia	1
Epistaxis	2
Bronchiectasis	1
Gonorrhoea, cystitis, and urethritis	1
Asthma	1
Fibrositis	1

* Excluding childhood infections.

fathers of mongol children only one had ANF-positive serum, and amongst 67 fathers of control (normal) children none had an ANF-positive serum. This population of men lived in the same area, were tested by the same laboratory technique, and had a similar mean age to the patients with tuberculosis surveyed. (The tuberculosis patients had a mean age of 45 and a range of 12 to 76 years.) The incidence of ANF positivity in the patients treated for tuberculosis by Cannat and Seligmann (1966) was 20%, and Alarcón-Segovia (1969) states: 'We have recently confirmed such higher incidence of antinuclear antibodies in isoniazid-treated tuberculosis patients.' Cannat and Seligmann (1968) were able to demonstrate the experimental induction of ANF by isoniazid in mice.

It would appear from the present survey that sex, acetylator phenotype, concomitant disease, and concomitant drug therapy (with isoniazid and PAS) are not related to the presence of ANF in patients treated for tuberculosis. Therefore it seems

reasonable to suspect that isoniazid is responsible especially since hydralazine (which, like isoniazid, is hydrazine with a substituent group on one nitrogen atom) causes ANF development in hypertensives.

There is a polymorphism in man for the metabolism of some drugs acetylated by hepatic N-acetyl transferase (Evans and White, 1964). Perry *et al* (1967) and Perry *et al* (1970) have shown that slow acetylators are more susceptible to the development of ANF, when treated for hypertension with hydralazine, than are rapid acetylators. The present survey indicates that the same does not hold true for the use of isoniazid in conventional doses for the treatment of tuberculosis.

Age emerges as a significant factor in the present survey in that ANF-positive patients are significantly older than ANF-negative patients. Cannat and Seligmann (1966) also found an increased incidence of ANF positivity in elderly (over 60) patients treated for tuberculosis. The presence of ANF is not a disease but can be considered a fore-runner of disease which may develop in some patients. (Alarcón-Segovia, 1969). It is possible, therefore, that the age-relationship of ANF development may be relevant to the known greater statistical predisposition of the elderly to adverse reactions to drugs (Hurwitz, 1969).

Summary

One hundred and three patients undergoing treatment for tuberculosis have been studied for acetylator phenotype and the presence of antinuclear factor in the serum. Seventeen patients were found to have antinuclear factor. The presence of antinuclear factor was not associated with either acetylator phenotype, sex, dosage of isoniazid, other disease concomitant with tuberculosis, or other

therapy concomitant with isoniazid and para-aminosalicylic acid. Patients with antinuclear factor were significantly older than those without.

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